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Ethnic Differences in Low Renin Status, Vascular Function and Renal Salt Handling

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**Ethnic Differences in Low Renin
Status, Vascular Function and Renal
Salt Handling**

*REnoVascular function Ethnicity Renin and
Endothelial Dysfunction (REVERED Study)*

Department of Clinical Pharmacology

Division of Cardiovascular Medicine

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I. KCL DECLARATION FORM

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II. ABSTRACT

Sodium retention with suppressed plasma renin activity (PRA) is more common in black subjects of African ancestry compared to white subjects and is associated with an increased propensity to the development of hypertension. This thesis investigates whether such low renin status might be secondary to reduced availability of endothelium-derived or other peri-renal tubular source of nitric oxide (NO). The association of flow-mediated dilation (FMD, a measure of the vasomotor response to endothelium-derived NO) to PRA was examined in a group of asymptomatic subjects (n=143) of African/African-Caribbean (n=84, classified as “Black”) and White European (n=59) self-defined ethnic groups. The effects of a change in salt intake on FMD were examined in a subset of 21 subjects and effects of inhibition of NO synthase on urinary sodium excretion were examined in a sub-set of 40 subjects. FMD was positively correlated with PRA, independent of age, gender, Black/White ethnicity, 24-hour urinary sodium excretion and blood pressure (standardised regression coefficient, $\beta = 0.32$, $P < 0.002$; FMD, $4.2 \pm 0.8\%$ vs. $7.3 \pm 0.7\%$, in subjects with $PRA < 0.5$ and $> 1.0 \text{ ngml}^{-1}\text{hr}^{-1}$ respectively, $P < 0.0001$). Change in salt intake over a two-week period did not significantly influence FMD. N^G -monomethyl-L-arginine (L-NMMA) reduced fractional urinary sodium excretion whereas saline placebo was without an effect. The anti-natriuretic effect of L-NMMA was positively correlated with PRA ($P < 0.01$, $3.4 \pm 7.0\%$ % vs. $29.6 \pm 5.5\%$ reduction in fractional sodium excretion in subjects with $PRA < 0.5$ and $> 1.0 \text{ ngml}^{-1}\text{hr}^{-1}$ respectively, $P < 0.01$). These results suggest that, in predominantly

asymptomatic subjects, renal sodium excretion is regulated by endothelium-derived or other peri-tubular source of NO. Reduced NO availability may underlie sodium retention and increased vascular risk associated with the low renin phenotype. Treatment with NO donors and/or enhancement of endogenous NO availability might be beneficial in subjects with low renin status. Further studies are required to explore the association of NO availability with sodium homeostasis in different groups (e.g. those with hypertension) and to test effects of interventions to modulate NO availability on sodium homeostasis and blood pressure in subjects with a low renin phenotype.

III. ACKNOWLEDGEMENTS

The three years spent in doctoral study have been both a challenge and an intellectually rewarding experience. The completion of this thesis has been met with several challenges that have both strengthened me both as an academic and a person.

I wish to express my sincere gratitude and appreciation to the Department of Clinical Pharmacology at St. Thomas' Hospital. I have made not only new colleagues in this journey but also new friends! In particular, I wish to thank my supervisors Professors. Chowienczyk and Ritter for all of their guidance and support both inside and outside of this work. I have been fortunate to have worked with both of them and look forward continuing collaborative work with the department.

To all my friends and family who have endured this journey with me, this work is as much your sacrifice as it is mine.

Finally, I wish to thank Him who has strengthened me continually through this life and looking forward to His continued blessings.

Dedicated to Mum and Dad, with Love.

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VII. LIST OF ABBREVIATIONS

ABPM	Ambulatory Blood Pressure Monitoring
ADH	Antidiuretic hormone
ANG II	Angiotensin II enzyme
BHS	British Hypertension Society
cAI	Central Augmentation Index
CVD	Cardiovascular Disease
CO	Cardiac Output
CIMT	Carotid Intima Media Thickness
cPP	Central Pulse Pressure
cSBP	Central Systolic Blood Pressure
CAD	Coronary Artery Disease
CHF	Congestive Heart Failure
DBP	Diastolic Blood Pressure
DCT	Distal convoluted tubule
E_{Peterson}	Peterson's Elastic modulus
eNOS	Endothelial Derived Nitric Oxide Synthase
FMD	Flow Mediated Dilatation
GFR	Glomerular Filtration Rate
11 β HSD2	11 β -hydroxysteroid dehydrogenase type 2 enzyme
HDL	High Density Lipoproteins
iNOS	Inducible Nitric Oxide Synthase
IHD	Ischemic Heart Disease
JGG	Juxtaglomerular granular cells

LDL	Low Density Lipoproteins
L- NAME	N ^G –nitro- L arginine methyl ester
L-NMMA	N ^G - monomethyl L-arginine
LoH	Loop of Henle
MBP	Mean Blood Pressure
NOS	Nitric oxide synthase
nNOS	Neuronal Nitric Oxide Synthase
PAD	Peripheral Arterial Disease
pAI	Peripheral Augmentation Index
pSBP	Peripheral Systolic Blood Pressure
PRA	Plasma Renin Activity
pPP	Peripheral Pulse Pressure
PCT	Proximal convoluted tubule
PVD	Peripheral Vascular Disease
ROMK	Rectifying outer medullary K ⁺ channel
RAAS	Renin Angiotensin Aldosterone System
SBP	Systolic Blood Pressure
SBP2	Systolic Blood Pressure 2
SVR	Systemic Vascular Resistance
TAL	Thick Ascending Limb
UKPDS	United Kingdom Prospective Diabetes Study
VLDL	Very Low Density Lipoproteins

1. BACKGROUND

1.1 Epidemiology of Cardiovascular Diseases and Hypertension

In the last half century, and exponentially in the last two decades, the global focus on health and health care delivery has shifted from the infectious diseases, such as dysentery, rheumatic fever and polio, to chronic non-communicable diseases (NCDs); the so called “lifestyle diseases”. This transition has seen both the creation and implementation of several government funded organizations, such as the United States Centre for Disease Control and Prevention (CDC), aimed at changing regional, national and international policy to address the two-fold rise of cardiovascular disease (CVD) in the next decade (Kearney, 2005). The explosion of NCDs over the last decade has led to the establishment of global targets in the fight against the current trajectory; the World Health Organization (WHO) 2025 nine strategic targets is an example of such a policy (WHO Authors, 2013).

CVD accounts for the largest percentage of the NCDs (Lim, 2012). It therefore represents an enormous health and socioeconomic burden for both mortality, but more seriously morbidity, in terms of personal and financial cost. It is likely, therefore, to be the most substantial health challenge of our century (Yusuf, 2001; Reddy, 2004). In the USA, an estimated eighty million Americans (1 in 3 Americans) have at least one CVD (Greenlund, 2004). It is

estimated that this figure may be as high as 1 in 2 persons for Americans over 60 years of age (Mozaffarian, 2015). Myocardial infarction (MI) and cerebrovascular accidents (CVA) have substantially increased in both the USA and the UK, though concomitant improvements in health care delivery have led to a decline in the mortality associated with these conditions (Beaglehole, 2011). There has been, however, a noticeable increase in morbidity across all ages with increased mortality rates among people less than 40 years (Alwan, 2010). It is thought that this has resulted primarily from a concomitant increase in the traditional risk factors for CVD; hypertension, diabetes mellitus and hypercholesterolemia. Unidentified novel risk factors may also play a pivotal role in explaining the changing epidemiology of CVDs – including peripheral arterial disease (PAD) and coronary artery disease (CAD) (Ridker, 2001). This cascade of modifiable risk factors may be interacting with non-modifiable factors, such as age and ethnicity, to accelerate disease processes in particular groups with higher morbidity and mortality rates. It has been observed in both the United Kingdom and the USA that an ethnic disparity exists with minority ethnic groups, especially black Caribbean people, having higher rates of certain CVDs than their Caucasian peers (Cruickshank, 2001). This difference is unlikely to be explained solely by differential access to health care experienced by the middle to lower income (minority ethnic) groups. One key barrier to addressing the CVD challenge is the identification of particular differences in its epidemiology in minority ethnic groups. In 2005, the American Society of Hypertension (ASH) revised its definition of hypertension as a progressive cardiovascular syndrome with many causes that

result in both structural and functional changes in the heart and vascular system.

The prevalence of hypertension varied in one study between 23 – 52% between six middle-income countries (Basu, 2013). This is forecasted to result in the mortality of some 9 million people globally (WHO, 2013). Unfortunately, without targeted interventions aimed at reducing its prevalence, CVD is expected to have a significant impact on the socioeconomic development of societies worldwide (Kearney, 2005). This will be especially true for developing countries, including the Caribbean (Chaturvedi, 1993), which account for sixty five per cent of the global population (Lane, 2001) of people with hypertension; that is currently six hundred and thirty nine of nine hundred and seventy two million people. This unbalanced proportion further contributes to the disease burden for which reduced health budgets are disproportionately matched. These so called lifestyle diseases, are sometimes referred to as the “silent killers”, and are by far the number one cause of death in the Caribbean region. To this end, the interest in hypertension and CVD risk has lead to the establishment of several national and regional guidelines aimed at improving measures for their prevention and treatment. The region has also established the civil society interest group, the Healthy Caribbean Coalition, which was conceptualized out of the 2008 Port-of-Spain declaration. The latter was a regional statement by Caribbean member states aimed at addressing the increased burden of NCDs. The impact of lifestyle disease is also observed in the multi-ethnic area of South East London (Cappuccio, 1997). Current guidelines for the treatment of hypertension in the United Kingdom are driven

by the epidemiological data and take into account not only age, gender and additional risk factors like diabetes mellitus and hypercholesterolemia, but also the differences observed between ethnic groups. This stratification of risk and treatment options is likely to have significant impact on the cardiovascular health in all populations but especially in minority ethnic groups.

1.2 Epidemiology of Ethnic differences in CVD and Hypertension

It has been described that Africans and Afro-Caribbean people have a relatively higher prevalence of stroke and end stage renal disease than White Europeans despite having a lower incidence of coronary artery disease (Tillin, 2012). This ethnic difference may relate in part to the relative differences in risk factors between the two ethnic groups; a higher prevalence of both hypertension and diabetes is observed in the Black ethnic group. One study has also shown a significantly increased risk of glucose intolerance, diabetes and insulin resistance in Black and South Asian people living in the UK (Basu, 2013). Compared to White Europeans, people of Black Caribbean origin living in the UK have significantly lower concentrations of very low-density lipoproteins (VLDL), small dense low-density lipoproteins (LDL), triglycerides, and central obesity (Mora, 2007) and this difference has been observed as early as 5-6 years of age (Whincup, 2010). This contradicts the accepted paradigm that insulin resistance is associated not only with glucose intolerance but also central adiposity. Reduced rates of smoking and increased levels of high-density lipoproteins (HDL) have been calculated to reduce the risk of IHD by twenty five percent. This may however be negated by the increased risk of twenty five per cent observed in this ethnic group because of hypertension.

In contrast to the incidence of IHD observed in Afro-Caribbean and African origin populations living in the UK, the relative risk of stroke is thought to be 1.5 – 2 times greater compared to the general population, with the incidence

rates higher in Black Afro-Caribbean people in the UK compared to White UK nationals (Smeeton, 2011). This difference observed in the Black ethnic group appears to be independent of the origin of the host population; as shown with Afro-Americans born in the USA compared with those born in the Caribbean (Chaturvedi, 1993). Another explanation for the difference observed is not only the higher blood pressures seen in this group but also the reduced or absence of nocturnal fall in blood pressure – the so called “dipping”. This results in a whole day sustained exposure to higher pressures when compared to other ethnic groups (Cruickshank, 1980). One possibility is that the loss of “dipping” may reflect central arterial stiffening, measured as increased aortic pulse wave velocity (PWV). Ongoing work is testing this hypothesis; earlier data showed that PWV was powerfully predictive of mortality, independent of and even displacing blood pressure, including in these ethnic groups (Cruickshank, 2002; Vlachopoulos, 2010). This difference in stroke risk between Black and White people is true for all subtypes of stroke but is particularly pronounced for haemorrhagic stroke. The stroke register within one South East London community suggests an incidence rate ratio of 5.04; 95% CI, 2.54 – 9.97 for haemorrhagic stroke in Black versus White people (Cruickshank, 1980; Sharma, 1999). Haemorrhagic stroke is the stroke sub-type most strongly related to blood pressure and Black versus White differences in stroke risk may be explained in part by differences in the prevalence and severity of hypertension (Chaturvedi, 2003). The higher hypertension rates observed in black participants are associated with other CVD risk factors, which increase in prevalence with advancing age (Rapsomaniki, 2014)

The United Kingdom Prospective Diabetes Study (UKPDS trial group, 1994) showed lower rates of peripheral vascular disease (PVD), in particular peripheral arterial disease (PAD), in Afro- Caribbean people compared to the rest of the population. Such first generation migrants are now approaching the six and seventh decades of life and so complications of IHD, like congestive heart failure (CHF), are likely to have a major impact on both mortality and morbidity.

Although several studies in the UK, Europe and North America report a higher prevalence of hypertension in Black vs. White people (Chaturvedi, 1993; Whitty, 1999) this ethnic difference was not observed in others (Manatunga, 1993; Cruickshank, 1983). In London, the best estimate of the prevalence ratio of hypertension in Black compared to White people is 2.6 (Meade, 1978). For Black people who have migrated to the UK, it is thought that an additional “migration factor” might play a role in influencing the statistics (Tillin, 2012). This difference, not only in the rate of hypertension, but also the type of end organ damage, has led to significant differences in the morbidity and mortality of CVD. In particular, the type of hypertension, low renin hypertension, observed more frequently in this group may account for the major differences observed across the cardiovascular disease continuum, as outlined next.

1.3 Renin and Salt Homeostasis

As mentioned in 1.2, the prevalence of low renin hypertension in Black people living in London is higher when compared to white people. Renin is a polypeptide hormone secreted by the kidney in response to physiological changes in sodium concentration (Sancho, 1976). The renin angiotensin aldosterone system (RAAS) plays a pivotal role in sodium homeostasis by regulating its excretion by the kidneys (Poch, 2001). It does this, in part, in response to the sodium concentration and systemic pressure detected by the kidneys (Weir, 1999). The plasma concentration of sodium is therefore regulated to remain between physiological limits of 135 – 145 mmol/L. The significance of this is probably not only related to the risk associated with hypertension but also the likelihood of accelerated arteriosclerosis through a salt mediated mechanism (Brewster, 2003). As sodium is consumed in the diet, mainly NaCl, a negative feedback mechanism triggers a fall in renin secretion. To understand how this system works, knowledge of the physiological handling of sodium by the kidneys and its functional unit, the nephron, is required.

1.3.1 The Nephron and Salt Handling

The kidney nephron is the functional unit of filtration and represents a sophisticated system of blood capillaries and tubules producing an ultra-filtrate which can then be further manipulated to produce varying concentrations of electrolytes. The highly convoluted structure of this delicate network of blood vessels allows for an increased surface area for the filtering of blood under pressure in the cup-like structure of Bowman's capsule as the glomerulus. The

kidneys produce, on average, approximately 180 L of filtrate, which after reabsorption of water and solutes produces 1.5 L of urine per day. A diagram of the process is illustrated in **Figure 1**

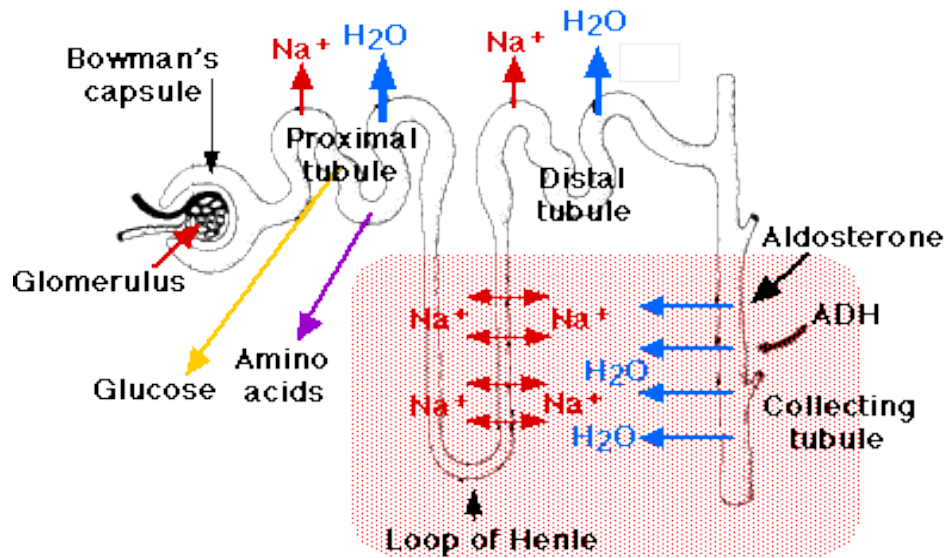


Figure 1: Diagram of the Nephron and Sodium Reabsorption sites
(From Berne & Levy Principles of Physiology 4th Edition)

Blood arrives at the glomerulus at the afferent arteriole and leaves this capillary tuft via the efferent arteriole. The system's two main properties are a relatively high surface area to volume ratio and a pressure difference across the afferent and efferent arteriole to produce the hydrostatic pressure required for filtration across the capillary basement membrane. The ultra-filtrate produced traverses the Bowman's capsule and continues distally through the proximal convoluted tubule (PCT); approximately sixty-five percent of the reabsorption of Na occurs here by active reabsorption via the Na⁺ - K⁺ ATPase sodium pump. This solute shift results in the iso-osmotic reabsorption of water, the end result being that more than seventy percent reabsorption of the ultra-filtrate occurs. The filtrate continues through the loop of Henle (LoH) where approximately twenty five percent of the filtered sodium is reabsorbed. In the thick ascending limb (TAL) of the LoH, a Na-K- 2Cl cotransporter ion channel is responsible for the reabsorption of Na back into the surrounding tubular space. This process is

dependent on the re-entry of K^+ back into the tubular lumen by another channel called the rectifying outer medullary K^+ channel (ROMK) and the intracellular Na^+ gradient generated by the interstitial Na^+-K^+ ATPase. The TAL is impermeable to water and so concomitant dilution of the tubular content occurs. This results in the concentration of filtrate as it flows down the descending limb of the loop that, unlike the TAL, is fully permeable to water. The filtrate then enters the distal convoluted tubule (DCT) where another ion channel, the Na-Cl cotransporter (NCCT), causes the further dilution of urine. The final regulation of Na occurs in the collecting tubules. Firstly, under the action of antidiuretic hormone (ADH) water is reabsorbed via transmembrane water channels called aquaporins that allow water to leave the lumen along its osmotic gradient. Secondly, Na reabsorption (2 – 3%) occurs across the epithelial sodium channel (ENaC). This channel is under the direct action of the mineralocorticoid aldosterone, which in turn is largely regulated by the renin angiotensin system (RAS) (Ritter, Textbook of Clinical pharmacology 5th Edition).

1.3.2 The Renin Angiotensin Aldosterone System (RAAS)

Renin is secreted in response to the relaxation of smooth muscle cells of the afferent arterioles of the kidneys in response to a decrease in glomerular perfusion pressure. There is a further sensing mechanism triggering renin release via the sensing mechanism of the macula densa cells of the juxtaglomerular apparatus to NaCl as shown in **Figure 2**. This results in renin release under conditions of reduced blood flow to the afferent arteriole and decreased NaCl concentration in the distal tubule.

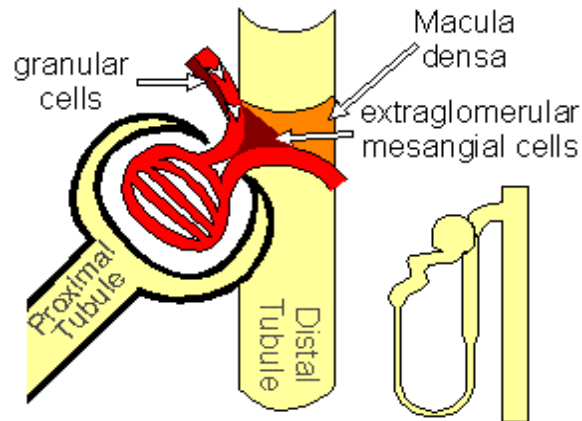


Figure 2: Diagram of the Juxtaglomerular Apparatus of the Nephron
(From Berne & Levy Principles of Physiology 4th Edition)

An outline of the RAAS is illustrated in **Figure 3**. Renin causes the cleavage of angiotensinogen to angiotensin I. The latter is then converted to its biologically active form as Angiotensin II. Angiotensin II, a potent vasoconstrictor, causes the release of aldosterone from the zona glomerulosa cells of the cortical adrenal gland. It also causes the release of ADH by the posterior pituitary gland, which results in the reabsorption of water from the collecting tubule. Both aldosterone and angiotensin II result in the reabsorption of Na from the DCT thereby decreasing the filtered load of Na. The increase in Na and water reabsorption results in an increase in the circulating plasma volume and this provides a negative feedback for renin release by increasing the renal perfusion pressure (Levy, Principles of Physiology 4th Edition).

The sodium channels in the DCT are now thought to be also under the direct influence of NO, as described in section 1.3.3, causing the excretion of Na and thus a counterbalancing effect to the processes described so far.

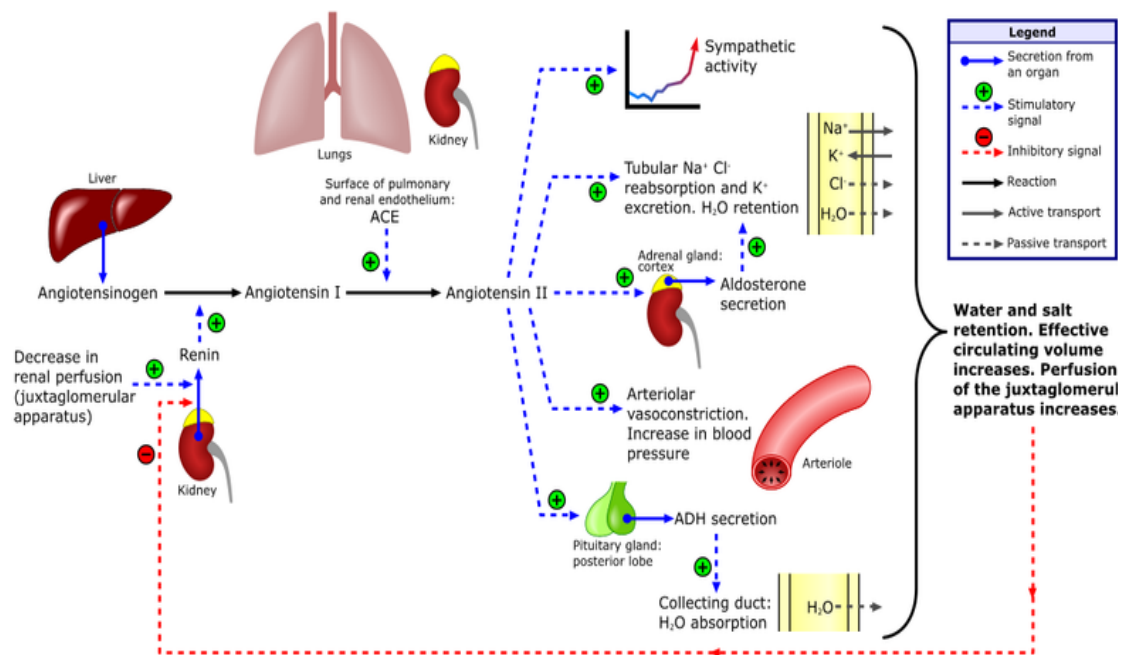


Figure 3: Diagram of the Renin Angiotensin Aldosterone System
(From Berne & Levy Principles of Physiology 4th Edition)

1.3.3 Nitric oxide and Sodium Excretion

It has been well appreciated that NO plays a critical role in the excretion of Na and water by the kidney by regulating glomerular filtration rate (GFR). It is, however, only recently that its role in the regulation of solute control, particularly Na, has been elucidated (Mattson, 1996; Stoos, 1995). In vivo experiments have shown that the stimulation of NO production results in an increase in the urinary Na content (Stoos, 1995), despite relatively little or no change in the GFR. It is now thought that NO produced by the TAL inhibits the luminal Na-K-2Cl cotransporter by inhibiting the chloride current (J_{Cl}) generated by this pump. The origin of the NO produced was further confirmed by the use of NO-specific measurements; NO-specific fluorescent dye and NO selective electrodes (Bachmann, 1994). It is still controversial as to the role of NO stimulation of ROMK, a channel allowing for the inward current of K into

the lumen, in the inhibition of the Na-K-2Cl co-transporter (Herrera, 2006). Prolonged (two hours) exposure of the energy dependent $\text{Na}^+ - \text{K}^+$ ATPase pump to NO resulted in a reduction in the pump activity by 32% but in vivo studies have not been confirmatory (Guzman, 1995). NO is also thought to increase the expression of the co-transporter in the TAL. One experiment has shown a reduction in Na-K-2Cl channel number after an infusion of N^G -nitro-L-arginine methyl ester (L – NAME), a NOS inhibitor. NO inhibition of the Na-K-2Cl co-transporter is thought to be mediated by its production of cyclic guanosine monophosphate (cGMP) (Guzman, 1995).

The NO produced by the TAL is thought to be due to the endothelial NOS isoforms (eNOS or NOS3) and this is also influenced by endothelin-1 binding to its receptor in the TAL; the ET_B receptor. A high salt diet has been shown to transiently increase (approximately 3 days) the level of NOS3 in the TAL with return to baseline after 28 days (Mattson, 1996). It is speculated that prolonged exposure to a high salt diet may lead to reduced levels of NO. Studies are still on going to ascertain the precise mechanism of the effect of a high salt diet on NO dependent inhibition of the Na-K-2Cl co-transporter.

1.3.4 Nitric oxide and Renin

Renin, a protease, is secreted from the juxtaglomerular granular cells (JGG) of the afferent arteriole of the glomerulus. These cells are thought to have developed from the morphological change of the arteriole's medial smooth muscle cells; a process, which is dynamic, and under the influence of Na concentration in the plasma, though the precise mechanism is not known

(Thorup 1996). The synthesis and secretion of renin is under the direct control of the rate of sodium intake, the renal perfusion pressure and the circulating levels of angiotensin II (ANG II). Under conditions of increased NaCl reaching the adjacent macula densa cells, there is an inhibitory signal to the JGG thereby decreasing renin release. This signal has not been identified but is referred to as the “macula densa signal” and is thought to be a critical inhibitor of renin release. There is a high concentration of both NOS1 (nNOS) and NOS3 (eNOS) in the macula densa cells and endothelial cells respectively. It is thought that NO may serve as a buffer in this tubuloglomerular feedback system as demonstrated in studies utilizing NO inhibitors where the NaCl – renin release phenomenon was impaired (Mundel, 1992). This influence appears to be related both to the dose and the duration of exposure to the inhibitor. There is also impairment of renin JGG production with the chronic inhibition of NOS3 by the macula densa cells. This was observed after chronic NOS inhibition caused significant reduction in the NOS3 gene and protein expression (Beierwaltes, 1995). This may explain the diminution in, not only salt excretion, but also vascular tone and haemodynamics observed in low renin states, since NO is known to be a key player in both events (Beierwaltes, 1995). Alternatively, rather than a direct influence of NO on renin release, the influence of NO on the Na-K-2Cl co-transporter could influence renin indirectly as discussed in 1.3.3.

1.4 Vascular Haemodynamics and Hypertension

1.4.1 Nitric Oxide and Endothelial Function

The endothelial cell layer represents a dynamic biologically active layer under the direct control of various substances (Tolins, 1991; Shepherd, 1991). Nitric oxide is one such biologically active molecule concerned with the modulation of arterial tone. To this end, the molecule is produced by the endothelium and has both an autocrine and paracrine mode of action (Fosteran, 2006). Vascular tone is regulated, in part, by factors causing vasodilatation and those causing vasoconstriction. Endothelial cells produce NO by means of endothelial nitric oxide synthase (eNOS or NOS3). The presence of the enzyme is therefore of critical importance in the regulation of basal vascular tone. Under the action of eNOS, NO and L-citrulline are produced from the amino acid L-arginine. The release of NO by the endothelium is triggered by increases in the shear stress on the endothelium as shown in **Figure 4**. NO plays a critical role in maintaining vasodilation in the basal state and during exercise (Kalliokoski, 2006). Vascular NO causes vasodilation through cGMP mediated smooth muscle relaxation. Two other isomers of NO have been discovered to date: inducible nitric oxide synthase (iNOS or NOS2) and neuronal nitric oxide synthase (nNOS or NOS1) (Shah, 2000). NO not only serves as a vasodilator, but also has a number of potentially anti-atherogenic actions such as inhibition of platelet activation, inhibition of the expression of adhesion molecules and inhibition of the proliferation of vascular smooth muscle (Lloyd-Jones, 1996; De Caterina, 1995)

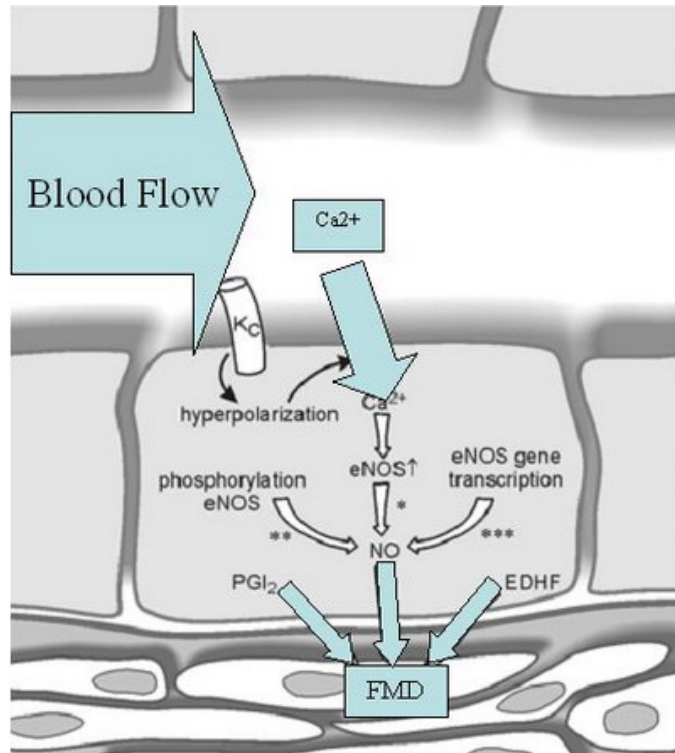


Figure 4: Diagram showing Ca^{2+} and NO mediated FMD
(Textbook of Human Physiology 10th Edition)

1.4.2 Endothelial Dysfunction in Hypertension and Atherosclerosis

Endothelial dysfunction is said to occur when there is a disruption of the balance between endothelium-derived vasodilation and vasoconstriction. There is evidence that this might occur in both HTN (Bolad, 2005) and in atherosclerosis (Charakida, 2006). In spontaneously hypertensive rats it has been shown that there is decreased expression of eNOS and hence a blunting of the NO-cGMP mediated relaxation of blood vessels. Atherosclerosis results from a cascade of events beginning with endothelial dysfunction and concluding with the production of an atherosclerotic plaque. There is decreased expression of eNOS in humans with developed atherosclerosis (Charakida, 2006; Napoli, 2001) and reduced bioavailability of NO is thought to underlie

the process of atherosclerosis. Hypercholesterolemia, one of the known risk factors for atherosclerosis, is associated reduced availability of endothelium-derived NO and this is also associated with other risk factors including diabetes mellitus and chronic cigarette smoking (Li, 2014).

1.4.3 Increased Arterial Stiffness: Increased Peripheral and Central Systolic BP

Arterial stiffness is a poorly defined entity in the scientific literature. Conduits that are described as “less stiff” are described as having a greater degree of compliance. Compliance is defined as the resultant change in volume (V) for an accompanying change in pressure (ΔP) (Sharma, 2012). Such that:

$$\text{Compliance} = \Delta V / \Delta P$$

Blood vessels are described as distensible (or less stiff) when there is a greater fractional change in the diameter (D) caused by a given pressure (Nichols, 1998):

$$\text{Distensibility} = \Delta D / (D \cdot \Delta P)$$

The inverse of this equation is a measure of arterial stiffness and is defined as the Peterson’s elastic modulus, E_{Peterson} (Peterson, 1960):

$$E_{\text{Peterson}} = (D \cdot \Delta P) / \Delta D$$

The stiffness of the artery is therefore not only related to the volume of blood transported but also intrinsic properties of the arterial wall (Gosling, 2003). The latter undergoes physical changes as a result of athero- and arterio-sclerosis, two distinct pathological processes, which have the effect of increasing arterial stiffness. Ang II causes the proliferation of vascular smooth muscle cells and causes the release of aldosterone by the adrenal glands (Brasier, 2002). The

latter is thought to play a significant role, not just in DCT salt handling, but also in inducing vascular fibrosis through collagen synthesis and hence arterial stiffness (Rehman, 2001).

Pulse Wave Velocity (PWV) has been accepted as the most practical method of assessing arterial stiffness and is employed in both research and clinical practice (as described in Chapter 4). PWV is based on the principle that stiffer arteries transmit the pulsatile components of pressure more rapidly due to decreased compliance of the vessel wall (O' Rourke, 2002).

1.4.4 Increased Wave Reflection: Increased Peripheral and Central Systolic BP

During ventricular contraction, blood leaves the left ventricle, with a defined pressure; along the arterial tree as an incident pressure wave (O'Rourke, 2002). The arteries in turn produce a reflected wave back to the left ventricle as a consequence of their limited distensibility, but also their progressively narrowing diameters towards the peripheral vasculature. Reflections were thought to occur most prominently at major junctions – e.g. the aortic bifurcation, major arterial branching angles etc. but may occur at other sites (O'Rourke, 2002). Both the amplitude and the time of arrival of the reflected wave on the incident wave affect the resultant pressure wave and hence blood pressure. In younger people, the reflected wave arrives back later in the cardiac cycle, during ventricular relaxation (diastole) and aids in the perfusion of the coronary arteries. With stiffer arteries, the reflected wave superimposes on the incident wave much earlier (during contraction or systole) and augmentation of

the initial pressure wave occurs. The resultant pressure that the left ventricle is exposed to is called the central systolic pressure (cSBP) and this increases the earlier the reflected wave returns. Also, the speed at which this happens relates to the PWV and hence to the arterial stiffness. Arteries stiffen with increasing age and in hypertensive states (O'Rourke, 1982). Reflections are also thought to cause pressure differences between the "central" SBP measured at the aorta (cSBP) and the peripheral systolic pressure (pSBP) measured at the brachial artery.

1.5 Predisposing Factors to Salt Sensitive HTN

1.5.1 Factors underlying low renin hypertension and why renin is low in black subjects

Sodium intake

A low-renin state is associated with “salt-sensitivity”, a pre-disposition towards blood pressure increasing with sodium load or decreasing with sodium restriction although the concordance of these two conditions may differ according to ethnic group (Shimojo, 1995). Greater sodium intake in Black versus White people with concurrent suppression of the renin-angiotensin system could potentially account for low-renin hypertension in Black people. However, although accurate data on sodium intake at a population level are limited, sodium intake alone is thought not to account for low-renin hypertension. Rather, low-renin hypertension is thought to be due to increased renal sodium reabsorption (Lovati, 1999)

Increased sodium retention (genetic influences)

Several monogenic forms of low-renin hypertension such as Gordon’s syndrome, glucocorticoid-remediable aldosteronism and Liddle’s syndrome, all of which are characterized by increased renal sodium absorption in the distal tubule, suppress renin and lead to salt sensitive hypertension (Shimijo, 1995). Additionally, genetic variations in candidate genes impacting these pathways have been implicated in both systolic and diastolic hypertension in the Black Caribbean ethnic group (Fox, 2011). With the possible exception of Liddles’s

syndrome, these syndromes account for only a small proportion of low-renin hypertension, but they suggest that low PRA may be due to a compensatory suppression of the RAAS when renal tubular sodium absorption is increased due to an effect independent of the RAAS. One author considers that the lower PRA in black compared to white subjects does not reflect differences in dietary sodium intake but is part of a “corrective” mechanism designed to maintain sodium balance in the presence of an increased tendency for sodium retention in black people (Sagnella, 2001). A plausible explanation for the higher renal tubular sodium reabsorption in black people might be related to mutations of the genes encoding the amiloride sensitive sodium channel. In one London study, measurement of the Na channel coding T594M gene found significantly higher levels of a T594M polymorphism in women, with a significant association with hypertension when adjusted for age. The prevalence of the T594M mutation was also higher in black people with hypertension than those without (Baker, 1998). Another possible mechanism relates to diminished activity of the enzyme 11 β -hydroxysteroid dehydrogenase type-2 enzyme (11 β HSD2). This enzyme protects the aldosterone receptor from occupation by cortisol (Lovati, 1999). A decreased activity of 11 β HSD2 has been reported in white salt sensitive people, but the relevance of 11 β HSD2 activity to salt sensitivity in black people (in whom salt sensitivity is of greater prevalence) and whether it might explain ethnic differences in salt sensitivity and renin status is unknown (Melikian, 2005).

Whilst the above possibilities do not directly involve the RAAS, but rather an alternate mechanism that leads to compensatory reduction in PRA and hence the RAAS, it is possible that ethnic differences in PRA relate directly to differences in activity of the RAAS. Genetic studies have shown ethnic variation in restriction fragment length polymorphisms at the gene loci for renin (Barley, 1991) that could be implicated in lower PRA in black compared to white subjects. Price suggests that low PRA in black compared to white subjects could be due to over activity of the RAAS at the tissue level in the kidney (Price, 2002).

1.6 Summary of Introduction

Hypertension-related cardiovascular disease represents a significant health burden globally, especially in minority ethnic groups. The main contributor to this greater burden could be the differences observed in both the prevalence and type of hypertension observed in different ethnic groups. Salt sensitive hypertension and a low-renin salt sensitive status have been shown to be more prevalent in the Black subjects and might therefore offer an explanation for the higher incidence of hypertension-related CVD in this group. It is also appreciated that an additional cardiovascular risk due to reduced availability of endothelium-derived NO and impaired endothelial function may contribute to CVD morbidity and mortality in Black subjects. NO plays a direct role in sodium homeostasis and reduced levels may decreased sodium excretion by the nephron. It is possible, therefore, that reduced NO availability may be a common factor for the setting up of both endothelial dysfunction and sodium retention.

2. MAIN HYPOTHESES

Subjects of Black African or Black Caribbean ancestry are known to have reduced flow mediated dilation (FMD) a measure of nitric oxide (NO) availability, compared to White European subjects (Bild, 2002). They are also known to have increased sodium retention as reflected by low plasma concentrations of renin. NO may influence sodium homeostasis by a natriuretic effect to decrease renal tubular sodium reabsorption. Reduced NO availability may explain low renin and low FMD in Black African or Caribbean as compared to White subjects. My hypotheses are that:

- I NO availability, as determined by FMD, relates to renin independently of ethnicity and other conventional risk factors for cardiovascular disease including age, gender, blood pressure and serum lipids.
- II This association is driven by NO availability determining sodium homeostasis and renin status so that:
 - a) Modulation of sodium load and renin does not influence FMD.
 - b) Inhibition of NO synthase inhibits sodium excretion; inhibition of sodium excretion is lower in subjects with low compared to high renin.

3. AIMS & OBJECTIVES

AIMS

- I** To determine whether NO availability, as determined by FMD, relates to renin independently of ethnicity and other confounding factors.

- II** To determine whether other measures of vascular function relate to renin independently of ethnicity and other confounding factors.

- III** To determine whether this association is driven by NO availability determining sodium homeostasis and renin status such that:
 - Modulation of sodium load and renin does not influence FMD
 - Inhibition of NO synthase inhibits sodium excretion; inhibition of sodium excretion is lower in subjects with low compared to high renin.

OBJECTIVES

1. To perform a cross sectional study to examine the relationship between flow mediated dilatation (FMD, a measure of endothelium-derived NO availability) in the forearm and plasma renin activity in participants representative of the asymptomatic population in the UK, including black African, black Caribbean and white European subjects. A secondary objective will be to examine the relationship of measures of vascular structure (carotid intima-media thickness and pulse wave velocity) to ethnicity and plasma renin activity. The relationship between FMD and plasma renin activity will be examined using multivariate regression analysis incorporating ethnicity and other confounding variables such as age and gender, blood pressure and serum lipids. Hypothesis I predicts that FMD will relate to renin independent of ethnicity and other confounding variables.
2. To examine the influence of oral salt loading on FMD in a subset of low renin (< 0.3 ng/ml/hr) subjects selected from the cross-sectional study above. A two-phase randomised cross-over study will be performed where subjects will receive a low salt diet plus placebo salt load and a low salt diet plus salt load in random order. Blood pressure, plasma renin activity, isoprostanes (as a marker of oxidative stress) and FMD will be measured after each treatment phase of the study. The major outcome will be FMD, which will be compared on the low and high salt intakes. Hypothesis II(a) predicts that FMD will not be influenced by modulation of salt intake.

3. To examine the influence of the inhibition of NO synthase, using the NO synthase inhibitor L-NMMA, on urinary sodium excretion and whether the influence of L-NMMA on urinary sodium excretion differs according to plasma renin activity. The relation between the effect of a fixed dose of L-NMMA on fractional urinary sodium excretion and plasma renin activity will be examined using multivariate regression analysis. Hypothesis II(b) predicts that the anti-natriuretic effect of L-NMMA will be positively correlated with PRA.

4. GENERAL METHODS

4.1 Subject Recruitment

The experimental work described in this thesis was approved by the Guy's and St. Thomas's NHS Foundation Trust (GSTT) Ethics Committee. Participants were each assigned a unique code that was used in all databases to avoid linkage of personal identifiers to particular volunteers.

Asymptomatic volunteers were recruited using King's College London internal webmail and from other databases within the department of Clinical Pharmacology at St. Thomas' Hospital. Written informed consent was obtained from all participants before starting the study protocol.

A telephone-screening questionnaire was carried out to determine the suitability of potential participants for inclusion into the study. During the interview, suitable participants were asked to avoid strenuous exercise for 24 hours before their study visits and to avoid taking caffeine. They were informed of the necessity of the overnight fast (from 22:00hrs) to obtain fasting biochemistry the evening before the visit.

4.2 Determination of Office BP and 24hr Ambulatory Blood Pressure Monitoring (ABPM)

Seated office brachial artery blood pressure was measured in accordance with British Hypertension Society guidelines in triplicate using an automated oscillometric device (Omron® 705CP, Omron®, Matsusaka, Japan) (El Assad, 2003). Participants were comfortably seated in a warm, quiet consultation room for 30 minutes before the first blood pressure reading was taken. The bladder of the cuff was placed 2 – 3 cm above the antecubital fossa with the forearm supported to heart level (below this tends to overestimate the systolic BP while above this tends to underestimate it). Readings were taken five minutes apart and the average of three readings was taken as the seated office BP.

Twenty-four hour ambulatory blood pressure monitoring was performed using the Spacelabs® Medical 90217 Ambulatory BP Monitors. This device has been validated by both the BHS and JNC VII for the determination of ambulatory blood pressure monitoring. Twenty-four hour ambulatory BP monitoring has been shown to be a better predictor of cardiovascular risk than the accepted office BP measurements. Firstly, it can identify those people who might have a unrepresentatively high office BP because of the psychological stress associated with the office visit, so called “white coat hypertension”. Secondly, it identifies those whose office readings are within the normal reference ranges but who may be above limits when carrying out their usual 24-hour routine. The latter is often referred to as “masked hypertension”. It also provides values of blood pressure during the night that some studies have shown to have greater

prognostic value than day-time blood pressures (Faggard, 2008). The absence of the physiologic fall in BP during the night has also been associated with higher CV events rates – including stroke (Ben-Dov, 2007).

4.3 Pulse Wave Analysis: Central Systolic Blood Pressure and Augmentation Index

Peripheral systolic blood pressure measured in the upper limb in the usual way as described above is amplified above central systolic blood pressure (cSBP) in the aorta by pulse wave reflection in the upper limb. This amplification depends not only on characteristics of the upper limb but the character of the central pressure waveform and hence on pressure wave propagation and reflection within the systemic circulation. Differences between peripheral SBP and cSBP are typically in the order of 10 mmHg but increase with increasing heart rate and/or peripheral vasodilation and may exceed 50 mmHg (Kroger, 1955). cSBP may be more closely related to cardiovascular outcomes than pSBP (Vlachopoulos, 2010) and anti-hypertensive drugs may have differential effects on cSBP and pSBP. In the CAFE study, for example, the intervention producing lower cSBP (but similar pSBP) was associated with improved outcome (Williams, 2006). cSBP is usually estimated by application of a “generalised transfer function” (GTF) applied to a peripheral pressure waveform (Chen, 1997). This approach has been criticised as dependent on limited and arbitrary “training” data from which such GTF are derived since these data may not be representative of subjects to which the GTF is ultimately applied (Hope, 1997). An alternative approach identifies the pressure at the late systolic shoulder of a peripheral pulse (pSBP₂, **Figure 5**) which has empirically been found to approximate cSBP (Pauca, 2004; Munir, 2008). An explanation for this has been provided in terms of wave reflection theory by Hughes et al: pSBP₂ is a point of inflection where the rate of change of pressure is zero. Wave intensity

(the product of rate of change of pressure and flow) is, therefore, also zero. The intensity of the backward travelling compression wave that causes peripheral amplification is then close to zero and peripheral and central systolic pressures are equal (Hughes, 2008). This approach has been validated (Guilcher, 2011) and is the method used in this thesis.

pSBP₂ is also used to derive the peripheral augmentation index (pAI), an index which is closely related to central augmentation index (cAI) and is thought to quantify pulse wave reflection within the arterial tree (Nichols, 1998; Melolenovsky, 2007) AI was thought to be related mainly to the timing of pressure wave reflection and therefore to be closely related to aortic pulse wave velocity (PWV). However, it is now acknowledged that AI can be changed independently of PWV and it is regarded as a composite index of wave reflection dependent on peripheral arterial tone and arterial stiffness (Kelly, 2001). It may also be influenced by ventricular ejection characteristics and its exact determinants remain to be established.

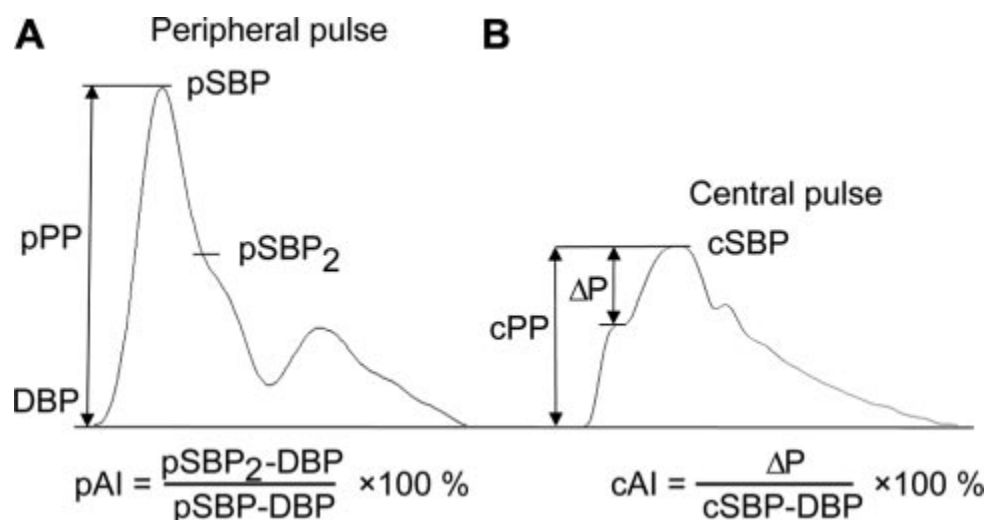


Figure 5: Diagram showing Peripheral and Central Pulse Waveforms

Peripheral pressure waveforms may be obtained using a device called the Finometer (Finapres Medical Systems). This system has been previously validated with the more widely used SphygmoCor system (Millasseau, 2000). A finger probe was placed on the index finger of the left hand which is approximately 10 cm below the level of the heart. Peripheral BP readings were taken with the Omron® 705CP and entered into an in-house software programme for the analysis of the waveform. Three pulse wave analysis readings were taken, each with repeat blood pressure measurements. Records of the SBP, DBP, pSBP2, and pAI were taken and mean results were taken for the three readings.

4.4 Pulse Wave Velocity

Arterial stiffness can be assessed using the velocity of pressure propagation along the arterial system (O'Rourke, 2002). A time delay occurs between the arrival of the wave impulse to the carotid or brachial artery and that arriving at the femoral artery. This time represents the transit time (TT) for the wave propagation between these two points. The pulse wave velocity (PWV) between these two surface points can therefore be estimated using the formula:

$$\text{PWV} = L/\text{TT}$$

where L is the path length between the two sites (e.g. carotid and femoral arteries) and can be estimated from surface markings. Pulse wave velocity was measured using the Vicorder® device (Skidmore Medical). The device utilises two cuffs that are separately applied to the arm and thigh. The pressure in the cuffs reaches 65 mmHg on cuff inflation. The distance between the arm and thigh cuffs is measured and entered into the programme as L and PWV is calculated based on the TT. The acquisition of the measurements using the device software is shown as a screen shot in **Figure 6**. Three values were taken and the average PWV was calculated.

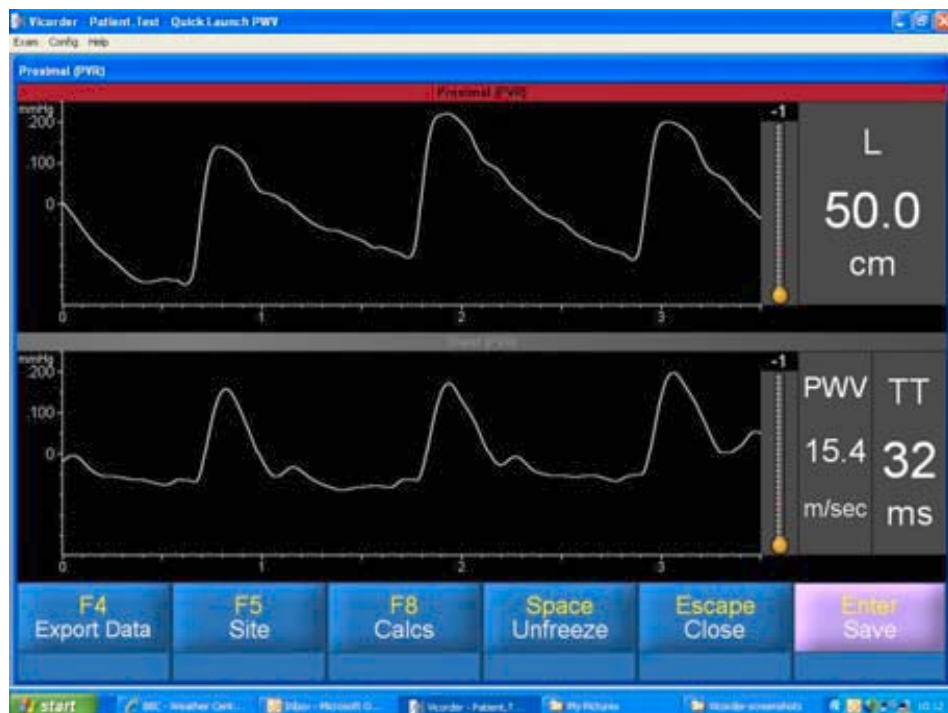


Figure 6: Diagram showing arterial waveforms for determining PWV using the Vicorder®

4.5 Flow mediated Dilatation

Flow mediated dilatation was used to assess endothelial function (Davignon, 2004). Two-dimensional longitudinal images were obtained of the brachial artery approximately 5 cm proximal to the antecubital fossa using the Acuson® 128XP ultrasound scanner equipped with a 7.5 MHz linear array transducer. A cuff was placed 3 cm distal to the antecubital fossa and was inflated to 250 mmHg for 5 minutes. Images were taken at baseline and for five minute after cuff release. The images were captured by trigger on the R wave of the continuous ECG monitoring representing end diastole. **Figure 7** shows a cross sectional view of the brachial artery during FMD scanning. Images were digitized for subsequent blinded analysis using automated edge detection software (Brachial Analyser, Medical Imaging Applications, LCC, Iowa, USA). After 10 min, baseline images were again repeated for one minute and endothelium-independent vasodilatation was assessed by administering 25 µg of GTN sublingually. Dilation to NTG (NTGD) was expressed as the percentage increase in brachial artery diameter from baseline to the maximal dilation after NTG.

FMD was calculated as below:

$$\text{FMD} = \text{Maximum Change in Diameter (mm)} / \text{Baseline Diameter (mm)} \times 100$$

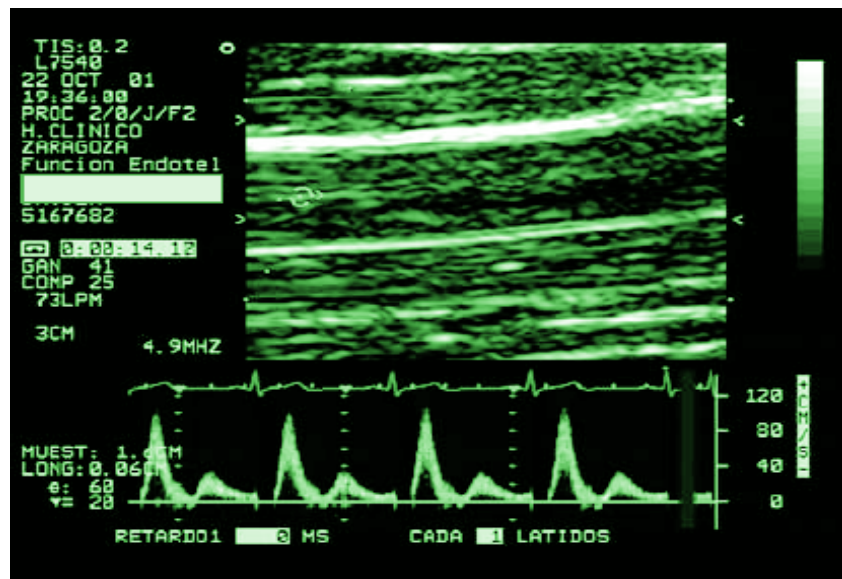


Figure 7: Showing FMD measurements of the brachial artery

4.6 Carotid Intimal Media Thickness (CIMT)

High-resolution ultrasound (Siemens CV70) was used to obtain images of the common carotid artery. Mean common carotid intima-media thickness (CIMT) was assessed from digitized images obtained in diastole of the near and far walls of both common carotid arteries 1-2 cm proximal to the flow divider (Mancini, 2004; Bots, 1997). This method is recognized as one of the most robust measures of IMT since the reproducibility of common carotid IMT measurements is higher than that of other segments, and in most studies to date additional measurements from the bifurcation and internal carotid has not improved the predictive value of IMT (O'Leary, 1999). The presence of plaque (defined as a focal widening relative to adjacent segments with protrusion into the lumen) within the near and far walls of the common carotid, bifurcation and internal carotid of both right and left arteries was scored according to the total number of plaques (giving a score of 0-12) (Sorke, 2004). **Figure 8** shows images of the longitudinal view of the common carotid artery just distal to the bulb.

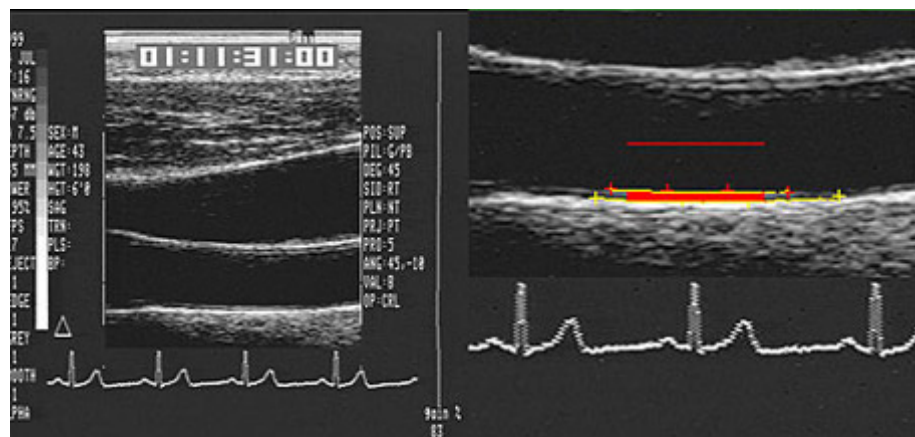


Figure 8: Photograph of the longitudinal section of common carotid artery measuring CIMT.

4.7 Biochemical Assays

Plasma renin activity (PRA) and aldosterone were measured at St Mary's Hospital, London. PRA was determined using a modification of the method described by Menard et al., a radio-immunoassay being used to measure angiotensin I generated from plasma incubated with endogenous plasma angiotensinogen substrate (Menard, 1972). The coefficient of variation (CV) for between-batch samples using this assay was 10.2% at a mean level of 1.26 ngml⁻¹hr⁻¹ with a lower limit of detection of 0.25 ngml⁻¹hr⁻¹. The distribution of plasma renin activity was skewed, with some values below the limit of detection. These values were set equal to the lower limit of detection. A radio-immunoassay was also used to measure plasma concentrations of aldosterone. The CV for between batch samples was 7.5% at a mean level of 22.2 ng/dl and the lower limit of detection 2.5 ng/dl. All other biochemistry assays were measured in Guy's and St Thomas' Hospital Chemical Pathology Laboratory using standard methodology.

4.8 Statistical Analysis and Sample Size calculations

Subject characteristics are summarised as means (standard deviation, SD) or as medians (inter quartile range) for non-normally distributed variables. Differences between ethnic groups were tested using a Student's t-test or Mann-Whitney test for non-normally distributed variables and Chi-squared test for categorical values. Results are summarised as means \pm standard error (SE). Associations between FMD (and other measures of vascular function), ethnicity and renin were examined using univariate and multivariate regression analysis with ethnicity (black or white) coded as a dummy variable. The influence of altering dietary salt intake on FMD was examined using analysis of variance for repeated measures (ANOVA). The influence of NO synthase inhibition on sodium excretion was examined using a paired Student's t-test. The relation of the amount of inhibition of sodium excretion produced by NO synthase inhibition to ethnicity and renin status was examined using multiple regression analysis. All analysis was done with SPSS version 19.

Sample size calculations

For the cross-sectional study comparing vascular measures across ethnic groups, a total sample size of 120 was estimated to give 90% power (with $P < 0.05$) to detect an effect of ethnicity or of plasma renin activity accounting for $> 10\%$ of the variance in the vascular measures when adjusting for age, sex, blood pressure and sodium intake. The sample size was calculated for hierarchical multiple regression assuming the addition of one variable accounting for $> 10\%$ of the variance in the vascular measures to an existing 5 variable.

An a-priori Sample Size Calculator for Hierarchical Multiple Regression Analysis was used. This software is now available in a web-based version from: <http://www.danielsoper.com/statcalc> (Soper, 2014)

In the salt intervention study, a sample size of 20 was estimated to allow a difference in FMD of > 2% points with > 90% power to be detected between low and high salt intakes. This calculation was for a paired *t*-test assuming a within-subject standard deviation of 2% points in FMD.

In the NO synthase inhibition study, a sample size of 40 was estimated to give 90% power to detect an effect of plasma renin activity accounting for > 20% of the variance in the fractional excretion of sodium when adjusting for baseline values, age, sex and ethnicity using hierarchical multiple regression.

5. CROSS SECTIONAL STUDY

5.1 Background

Previous studies examining ethnic differences in renin status have mainly focused on hypertensive subjects, although studies in normotensive subjects do suggest that PRA is lower in normotensive as well as hypertensive black compared to white subjects (James, 1986; He, 1999). Similarly studies on ethnic differences in vascular characteristics have focused mainly on patients with hypertension or older subjects with risk factors for vascular disease. The aim of this study was to examine ethnic differences in salt intake, renin status and vascular structure and function in an apparently asymptomatic group of young adults of African/African-Caribbean and White ethnicity recruited from educational establishments in South London. Because of the possible relation between NO and sodium homeostasis discussed in Chapter 1, a specific aim was to examine the relationship between flow-mediated dilation (this being largely determined by endothelium-derived NO) and plasma renin activity.

5.2 Participant Characteristics

Participants for this study were recruited using an advertisement on King's College London internal webmail and from databases in the Department of Clinical Pharmacology, St. Thomas' Hospital; participants were mainly staff or students of the college and other educational establishments. The advertisement made clear the rationale for the study and the requirement for inclusion of persons of African/African-Caribbean ethnicity aged between 18 and 50 years.

Towards the end of the study, these inclusion/exclusion criteria were relaxed to allow the inclusion of subjects up to age 60 in order to achieve the required recruitment numbers. The response to this resulted in an approximately balanced recruitment of persons of African, African-Caribbean and Caucasian self-defined ethnicity. Exclusion criteria included: BMI > 35 kg m⁻²; smoking within the last 9 months; diagnosis of diabetes mellitus, hypertension, cardiovascular disease, sickle cell disease (but not sickle cell trait) or any other significant condition; alcohol consumption more than 14 units/week for women and 21/week for men in keeping with the current National Health Service (NHS) UK recommendations. Subjects with undiagnosed hypertension found to be hypertensive in this study were not excluded but were referred to their General Practitioner for continued management.

5.3 Study Protocol

Participants were seen in the research facility on two consecutive study days. All participants received a reminder telephone call the evening before the first visit to remind them to fast overnight as well as the necessity for a 24-hour urine collection the following day. Participants arrived on the morning of the first day having completed an overnight (at least 12 hours) fast. Seated blood pressure measurements were then taken using an Omron® device after at least thirty minutes with the participant seated and this was repeated at five minute intervals for two more readings. The average of these three readings was used as the resting clinic blood pressure. Anthropometric measures: height, weight and waist and hip circumferences were recorded and BMI and waist to hip ratio calculated. Participants were asked to lie supine for a further ten minutes before the first supine reading of blood pressure was taken. Measurements were then taken of pulse wave velocity with the Vicorder® device; with appropriate cuffs placed on the right arm and thigh. Care was taken to ensure that the thigh cuff was applied as high up the thigh as possible with the participant's knee initially slightly bent. Three readings were then taken five minutes apart and an average reading was taken as the pulse wave velocity. With participants still supine, the Omron® blood pressure cuff was then applied to the left arm and the Finometer® finger probe was applied to the right index finger for the measurement of pulse wave analysis. Three consecutive readings were then taken and the average of these was used for the pulse wave analysis.

Ultrasound measurements of flow mediated dilation (FMD) and carotid intimal media thickness (CIMT) were obtained using the Siemens CV70. Images were digitalized for subsequent blinded analysis using automated edge detection software (Brachial Analyser, Medical Imaging Applications, LCC, Iowa, USA). CIMT measurements were taken first beginning with the left carotid artery in transverse section. For measurement of FMD, the participant's left arm was suitably positioned and a BP cuff was applied to the forearm just below the medial epicondyle. The ultrasound probe was then positioned over the brachial artery about 5 cm above the elbow. A baseline scan was taken for one minute with image storage for future analysis and printed copies taken for participant's printed records. The cuff was then inflated to a pressure of 250 mmHg while still scanning the brachial artery. This was left inflated for 5 minutes while still scanning the brachial artery. The cuff was then deflated and images were stored at 5, 10, 15 and 30 seconds with continuous scanning for another 5 minutes.

A further scan was taken at baseline for one minute followed by the sublingual administration of 25 mcg of glyceryl trinitrate (GTN). This was immediately followed by 5 minutes of further scanning. FMD was expressed as the percentage increase in brachial/radial artery diameter from baseline to maximal dilation that occurred 30 to 90 sec after the release of the cuff. Dilation to GTN (GTND) was expressed as the percentage increase in brachial artery diameter from baseline to the maximal dilation after GTN.

With patients remaining supine, venous blood was taken for the measurement of plasma glucose, electrolytes, creatinine, total-, LDL- and HDL-cholesterol, triglycerides, plasma renin activity and serum aldosterone measurement. Samples of serum and plasma were also stored at -80 C.

Participants were then given containers for the collection of a 24-hour urine collection and fitted with a twenty-four hour ambulatory blood pressure monitor (24h ABPM). Participants then left and returned the following day at the same time of day, having performed a 24h urine collection and having undergone the 24h AMBP. The total volume of urine was measured and a sample stored at – 80°C for future analysis. A sample was also sent to the clinical chemistry laboratory for the measurement of urinary sodium, potassium and creatinine concentrations. Twenty-four hour urinary sodium was then calculated from concentration and volume.

5.4 Results

5.4.1 Baseline Participant Characteristics

A total of 144 participants were enrolled in this cross sectional study. One participant withdrew due to unexpected relocation overseas, thus 143 participants completed the study. Eighty-four (59%) were black and of these 65% (55) were African while 35% (29) were of Black Caribbean ancestry. The remainder of the participants (59) was of white European ethnicity. Two black subjects and 5 white subjects were aged over 50.

Table 1 shows the characteristics of the participants categorized by ethnicity (described as black or white). There was no significant difference in the mean age or waist: hip ratio in the two groups but black participants had significantly greater BMI compared to white participants. There were no statistically significant differences in either systolic or diastolic blood pressures recorded on the clinic visit. There was, however, a statistically significant difference in the daytime systolic BP with higher day-time systolic BP in black compared to white participants ($P < 0.004$) but no significant difference in night-time systolic BP or in day or night diastolic BP (table 1). There were no significant differences in serum lipids between the two ethnic groups. Fasting plasma glucose was marginally but significantly higher in black compared to white participants ($P < 0.05$).

Table 1: Baseline Participant Characteristics in Cross-Sectional Study

Measure	ALL	Black	White	P value
n	143	84	59	
Age (years)	30 (10)	31 (10)	30 (10)	NS
BMI (Kgm ⁻²)	25.7 (4.5)	26.8 (4.5)	24.2 (4.0)	< 0.0001
Waist: Hip	0.79 (0.14)	0.80 (0.11)	0.79 (0.17)	NS
BP (mmHg)				
Seated Systolic BP	121 (12)	121 (12)	120 (14)	NS
Seated Diastolic BP	76 (9)	76 (9)	76 (9)	NS
ABPM Systolic (day)	122 (12)	125 (13)	119 (9)	0.004
ABPM Diastolic (day)	74 (8)	75 (8)	74 (8)	NS
ABPM Systolic (night)	115 (11)	117 (12)	112 (9)	NS
ABPM Diastolic (night)	67 (7)	68 (8)	67 (6)	NS
Total Chol. (mmol/L)	4.52 (0.99)	4.54 (1.00)	4.48 (0.96)	NS
LDL (mmol/L)	2.60 (0.88)	2.65 (0.91)	2.52 (0.83)	NS
HDL (mmol/L)	1.56 (0.43)	1.55 (0.40)	1.57 (0.49)	NS
TAG (mmol/L)	0.80 (0.49)	0.75 (0.43)	0.87 (0.45)	NS
Glucose (mmol/L)	4.6 (1.4)	4.6 (1.0)	4.8 (1.0)	< 0.05
Serum Na (mmol/L)	142 (2)	142 (2)	143 (2)	NS
Serum K (mmol/L)	4.1 (0.3)	4.2 (0.3)	4.1 (0.3)	NS
Urea (mmol/L)	4.7 (1.4)	4.6 (1.3)	4.7 (1.7)	NS
Creatinine (mmol/L)	80 (15)	84 (16)	75 (12)	0.001
PRA (ngml ⁻¹ hr ⁻¹) ¹	0.7 ¹ (0.6)	0.6 (0.6)	0.9 (0.6)	0.014²
Aldosterone (pmol/L)	176 (108)	171 (102)	185 (112)	NS
Urine Na: Cr	9.2 (3.9)	9.2 (3.3)	9.3 (4.7)	NS

Values are means (SD) except where noted. P values refer to differences between black and white subjects (unpaired t-test except where noted). BMI, body mass index; ABPM, ambulatory blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; TAG, triacylglycerol; Na, sodium; K, potassium; PRA, plasma renin activity; Urine Na:Cr, ratio of urine sodium:creatinine concentrations.

¹Median (inter-quartile range). ²Mann-Whitney U test.

Urinary Sodium excretion and Plasma Renin Activity in relation to ethnicity

24-hour urinary sodium/creatinine was similar in the two ethnic groups but PRA was significantly lower in black compared to white participants ($P=0.014$, by Mann-Whitney U test, **Figure 9**). When defined as $PRA < 0.3 \text{ ng/ml/hr}$, 54% of black participants had a low renin status as compared to 6% of white participants ($P < 0.001$). PRA was significantly correlated with age ($R = -0.426$, $P < 0.001$, **Figure 10**) but there was no significant correlation between PRA and 24-hour urinary sodium to creatinine ratio (**Figure 11**) or to 24 hour urinary sodium excretion ($P = 0.68$). When adjusted for age and urinary sodium/creatinine, PRA (log-transformed to achieve a normal distribution) was significantly lower in black compared to white participants.

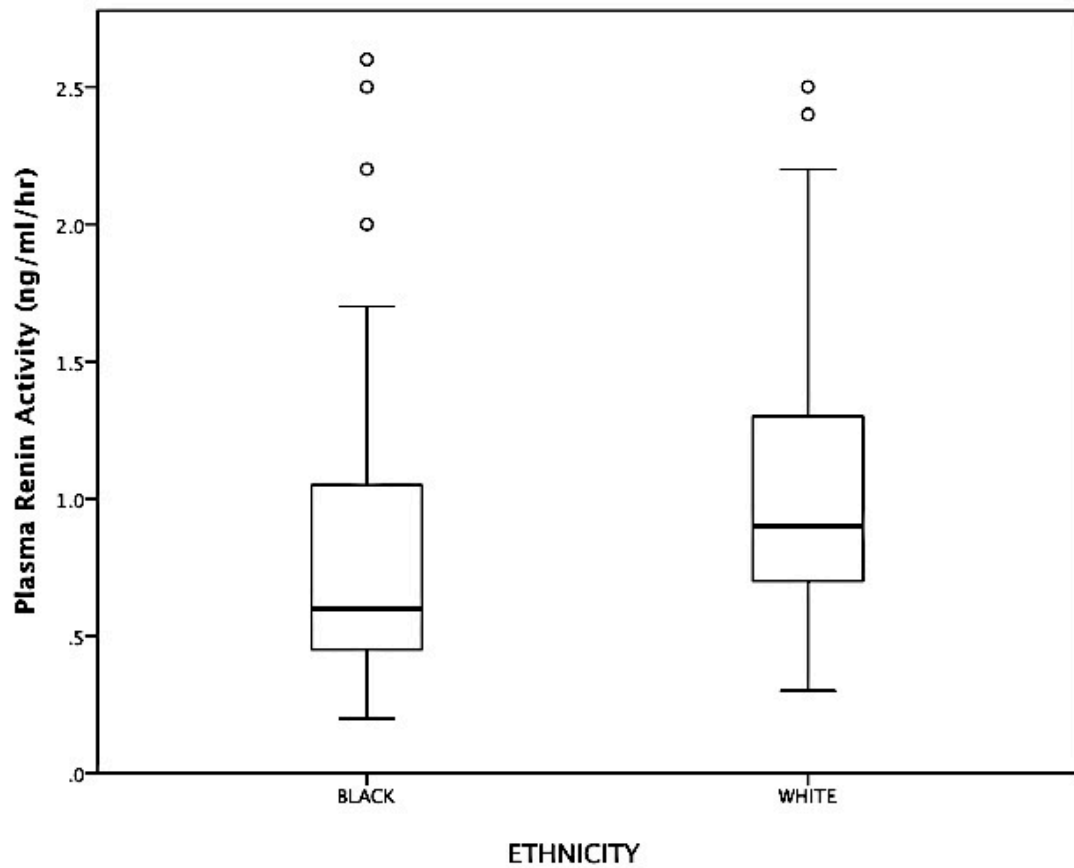


Figure 9: Box plot showing the distribution of PRA in the two ethnic groups. (n=84 and n=59 for black and white subjects respectively). The median values are shown (solid central horizontal lines) with the box marked by the first and third quartiles. The extension bars show the range of values for each group. Outliers are shown as circles.

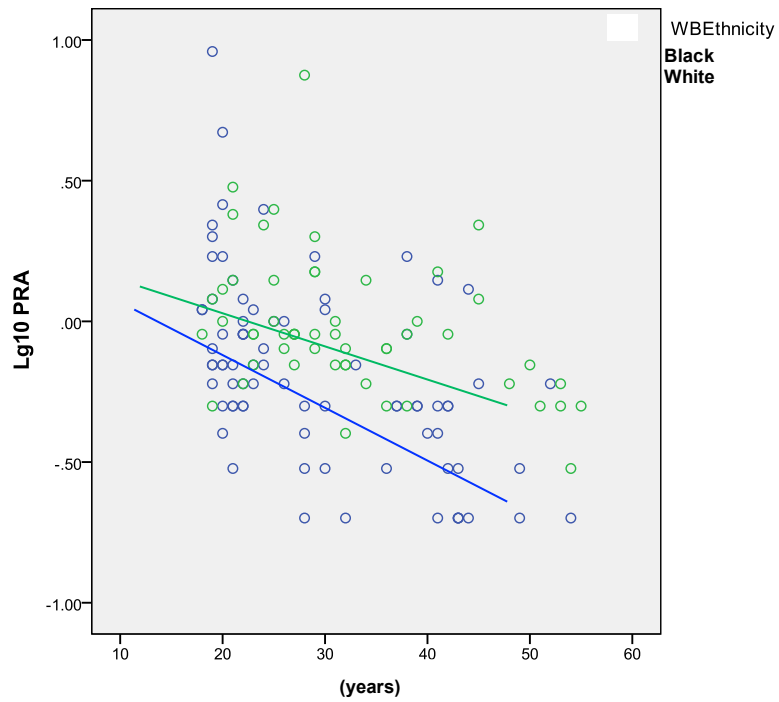


Figure 10: Scatter plot of PRA (log to base 10, Lg10PRA) versus age with subjects sub-divided by ethnicity (n=84 and n=59 for black and white subjects respectively).

The solid lines are regression lines for each group. ($R=-0.426$, $P < 0.001$, for all subjects).

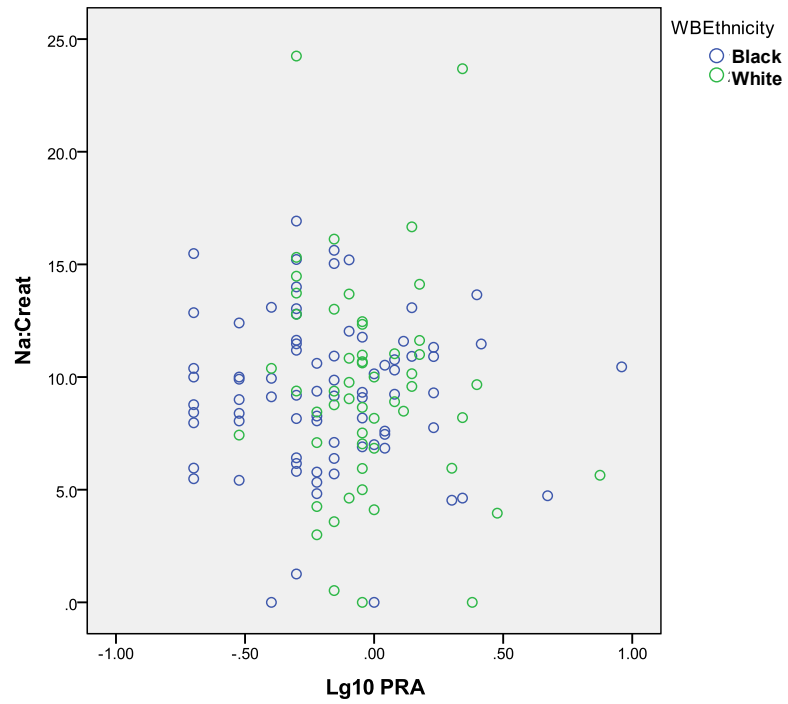


Figure 11: Scatter plot showing lack of relationship between urinary salt excretion as urinary sodium to creatinine concentration (Na:Creat) and PRA (log to base 10, Lg10PRA, with subjects sub-divided by ethnicity (n=84 and n=59 for black and white subjects respectively, $R = -0.052$, $P = 0.55$ for all subjects).

Vascular measures in relation to ethnicity

Measures of vascular structure and function for the two ethnic groups are shown in **Table 2**. There was a trend towards lower values of FMD (**Figure 12**) and higher values of PWV in black compared to white participants but this did not reach significance. AIx and GTND were not significantly different in the two groups. CIMT was, however, significantly greater in black compared to white participants. These findings were unchanged when adjusted for age, gender, blood pressure and other subject characteristics found to be significantly correlated with the vascular measures.

Table 2: Summary of means for vascular outcome measures according to ethnicity.

Measure	Black	White	P value
	(n=84)	(n=59)	
PWV (m/s)	10.1 (1.8)	9.9 (1.3)	NS
AI (%)	55.5(18.5)	53.8 (20.4)	NS
FMD (%)	5.9 (3.6)	6.8 (4.1)	NS
GTND (%)	9.8 (5.2)	10.1 (5.1)	NS
Average CIMT (mm)	0.48 (0.12)	0.44 (0.11)	< 0.005

Values are means (SD). P values refer to differences between black and white subjects.

PWV, Pulse Wave Velocity; AI, Augmentation Index; FMD, Flow Mediated Dilatation; GTND, percentage dilatation to GTN.

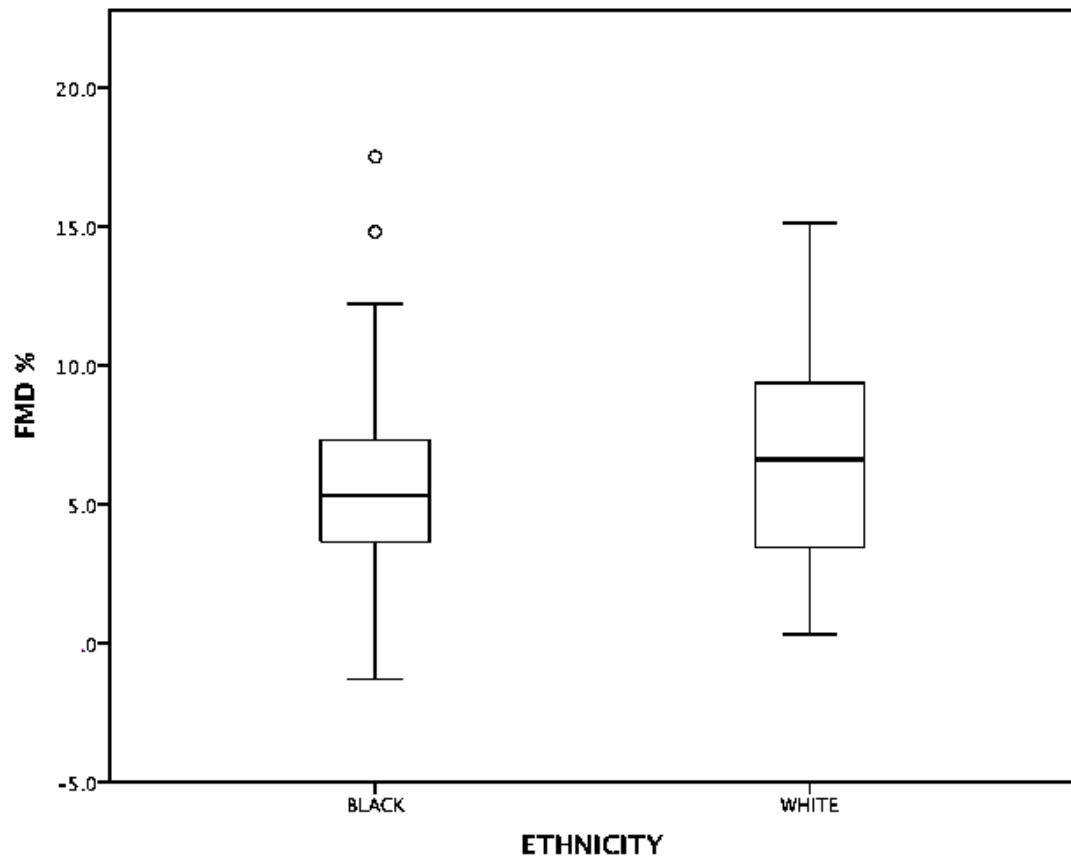


Figure 12: Box plot of FMD in black and white ethnic groups (n=84 and n=59 for black and white subjects respectively). The median values are represented as the bold horizontal line in each box with the 1st and 3rd interquartile ranges shown as the lower and upper borders respectively. The extension lines show the range of values for each group. Outliers are shown as circles.

Relation of vascular measures to participant characteristics and PRA

Results of univariate regression analysis examining the relation of each of the vascular measures to participant characteristics are shown in **Table 3**. FMD was weakly but significantly negatively correlated with night ambulatory systolic BP and strongly positively correlated with PRA (**Figure 13**). CIMT was significantly correlated with age, ethnicity, central SBP, daytime ambulatory SBP, HDL-cholesterol and PRA. PWV was significantly correlated with age, BMI, all BP components, LDL-cholesterol and negatively correlated with PRA. A1x was significantly correlated with age, male gender, central SBP, ambulatory DBP, LDL-cholesterol and negatively correlated with heart rate and PRA.

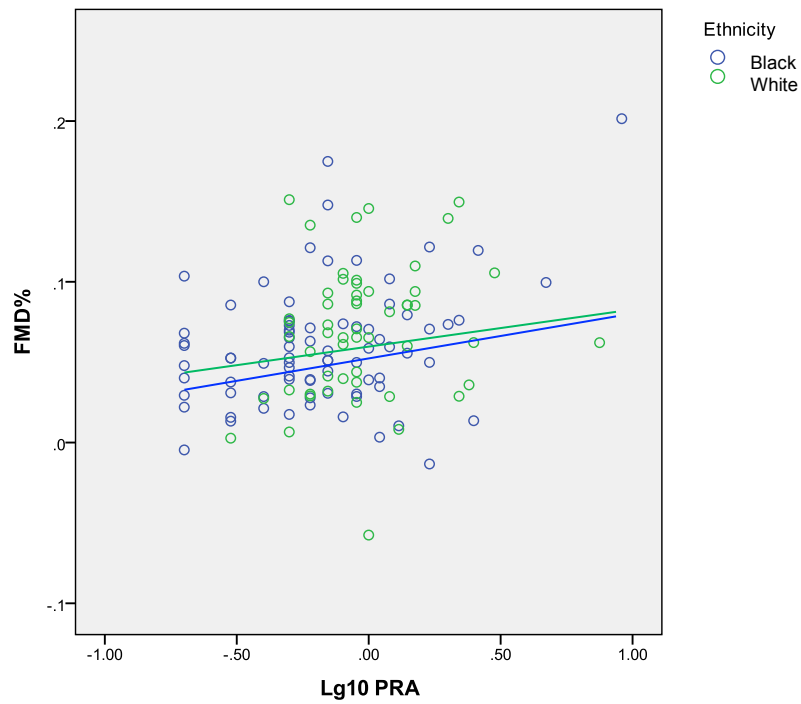


Figure 13: Scatter plot of FMD vs PRA (log to base 10, Lg10 PRA) with subjects sub-divided by ethnicity (n=84 and n=59 for black and white subjects respectively). The solid lines are regression lines for each group (R= 0.291, P<0.001 for all subjects).

Multiple regression (stepwise backward) analysis demonstrated that FMD was significantly independently correlated only with PRA in a model where age, gender, ethnicity, 24-hour urinary sodium and ambulatory night systolic blood pressure (the only variable significantly associated on univariate analysis with FMD apart from PRA) were entered. 24 hour urinary sodium was used in preference to sodium/creatinine ration because of the possibility of confounding due to differing lean body mass in black and white subjects. However, FMD remained significantly independently positively associated with PRA when either 24 hour urinary sodium: creatinine or 24 hour urinary sodium was forced into the model and irrespective of whether any or all of gender, age and ethnicity were forced into the model. CIMT was significantly independently correlated with age, ethnicity and central SBP. PWV was significantly independently correlated with age, BMI, night ambulatory SBP and central SBP. AIx was significantly independently correlated with age, gender, central SBP and negatively correlated with heart rate.

Table 3: Univariate regression analysis of FMD and CIMT versus age, gender, ethnicity and other subject characteristics significantly correlated with FMD and CIMT.

Dependent variable	Independent variable	R	P
FMD	Age	-0.05	=0.14
	Gender	0.08	=0.135
	Ethnicity	0.11	=0.18
	ASBP (night)	-0.17	<0.05
	PRA	0.20	<0.001
CIMT	Age	0.36	<0.001
	Gender	0.05	=0.57
	Ethnicity	0.21	<0.01
	cSBP	0.39	<0.001
	ASBP (day)	0.17	<0.05
	HDL	0.18	<0.05
	PRA	0.30	<0.001

R, Pearson's correlation coefficient; BMI, body mass index; cSBP, central systolic blood pressure; PRA, plasma renin activity; ASBP, ambulatory systolic blood pressure; LDL, LDL-cholesterol; HDL, HDL-cholesterol.

Table 4: Univariate regression analysis of PWV and AIx versus age, gender, ethnicity and other subject characteristics significantly correlated with PWV and AIx.

Dependent variable	Independent variable	R	P
PWV	Age	0.42	<0.001
	Gender	0.06	=0.47
	Ethnicity	-0.06	=0.50
	BMI	0.32	<0.001
	Waist:hip	0.20	<0.05
	cSBP	0.52	<0.001
	ASBP (day)	0.32	<0.001
	ADBP (day)	0.38	<0.001
	ASBP (night)	0.31	<0.001
	ADBP (night)	0.28	<0.001
	LDL	0.17	<0.05
	PRA	-0.29	<0.001
AIx	Age	0.63	<0.001
	Gender	0.35	<0.001
	Ethnicity	-0.04	=0.60
	HR	-0.30	<0.001
	cSBP	0.80	<0.001
	ADBP (day)	0.35	<0.001
	LDL	0.35	<0.001
	PRA	-0.36	<0.001

R, Pearson's correlation coefficient; BMI, body mass index; cSBP, central systolic blood pressure; PRA, plasma renin activity; ASBP, ambulatory systolic blood pressure; ADBP, ambulatory diastolic blood pressure; LDL, LDL-cholesterol; HDL, HDL-cholesterol.

Table 5: Multivariate regression analysis of FMD, CIMT, PWV and AIx versus variables significantly correlated on univariate analysis.

Dependent variable	Independent variable(s)	β	P
FMD	PRA	-0.28	<0.001
CIMT	Age	0.27	<0.001
	Ethnicity	0.21	<0.01
	cSBP	0.27	<0.02
PWV	Age	0.24	<0.01
	BMI	0.15	<0.05
	ASBP (night)	0.24	<0.01
	cSBP	0.27	<0.01
AIx	Age	0.19	<0.01
	Gender	0.20	<0.001
	HR	-0.16	<0.01
	cSBP	0.60	<0.001

b, standardized regression coefficient; BMI, body mass index; cSBP, central systolic blood pressure; PRA, plasma renin activity; ASBP, ambulatory systolic blood pressure; HR, heart rate.

5.5 Discussion

Ethnic differences in Plasma renin activity

As described in the introduction, it is well appreciated that there are ethnic differences in renin status in hypertensive subjects, with a greater prevalence of low PRA in black compared to white subjects. Less is known about renin status in normotensive subjects. However, the findings of this study confirm findings in other population studies (Sagnella, 2001) that, even in predominantly normotensive subjects, PRA activity is lower in black compared to white subjects and hence the prevalence of “low renin” status is greater in black compared to white subjects. Suppressed PRA activity could be due to increased salt intake (He, 2001) and/or increased renal sodium reabsorption. There are difficulties in estimating salt intake since 24 urinary collections may be incomplete and attempting to normalize this by using a sodium: creatinine ratio may be confounded by ethnic differences in lean body mass. However, there was no significant correlation of PRA with either measure of urinary sodium excretion. The lack of correlation between PRA and urinary sodium does not mean that dietary sodium does not influence PRA, since a dietary intervention in which there is a large change in salt intake does influence PRA as will be demonstrated in Chapter 6. However, lack of a correlation of PRA with urinary sodium in the present study, in which the range of salt intake was restricted to that due to usual variation in dietary intake, is consistent with the established view that ethnic differences in renin status relate primarily to renal sodium handling rather than salt intake (Bochud, 2009). It is notable that in this predominantly normotensive group ambulatory daytime systolic blood pressure was higher in black compared to white subjects.

Ethnic differences in vascular structure and function: FMD

There is relatively little data on ethnic differences in FMD from previous studies, which have been limited to small sample sizes and/or included patient populations. One study compared FMD in 28 young asymptomatic black African-Americans and 24 white Caucasian subjects and demonstrated lower FMD in black compared to white subjects ($1.76 \pm 0.56\%$ in black compared to $8.79 \pm 1.22\%$ in whites subjects, $P < 0.001$) (Perregaux, 2000). Similarly Melikan et al demonstrated lower FMD in a group of 30 young black African men compared to that in a group of 28 white European men in the UK, although the difference (5.2 ± 0.3 vs $6.3 \pm 0.4\%$ in African vs. white European) was less marked than in the study by Perragaux et al (Melikan, 2007). By contrast, in a larger group of 228 black and white Americans, approximately 50% of whom were hypertensive, Gokce et al demonstrated no difference in FMD between black and white subjects (Gokce, 2001). However, in this study, conduit artery vasodilation to GTN was enhanced in black compared to white subjects. In the study described in this chapter, in a larger sample size than that for previous studies in asymptomatic subjects, I found a non-significant trend towards lower FMD in young black African and African-Caribbean subjects compared to white subjects. Thus the available evidence suggests the FMD is similar or reduced in black compared to white subjects, with the possibility that differing findings in studies to date relate to the characteristics of the subject groups and limitations of self-defined ethnicity in capturing the genetic and environmental components which may influence vascular phenotypes.

Ethnic differences in vascular structure and function:

CIMT

By contrast to FMD, CIMT represents a structural change in the wall of the artery that may represent hypertrophy of vascular smooth muscle in response to vascular risk factors, particularly hypertension, and/or early change in the intima associated with sub-clinical atherosclerosis. In a population study of middle aged subjects in South London, Mackinnon et al found greater CIMT but interestingly lower prevalence of carotid plaque in black compared to white subjects (Mackinnon, 2010). Whincup et al have reported greater CIMT in children of black African compared to those of white European origin (Whincup, 2012). The findings of my study are in agreement with these previous studies with significantly higher CIMT in young black African/African-Caribbean subjects compared to white subjects in South London. The probability of atherosclerosis is low in the relatively young subjects in my study and in the children studied by Whincup et al. This and the presence of higher CIMT but lower risk of sub-clinical atherosclerosis as determined by focal protrusion of plaque into the vascular lumen in black compared to white subjects in the study of Mackinnon et al suggests that higher CIMT in black compared to white subjects is likely to be due to relative hypertrophy of vascular smooth muscle rather than thickening of the intima as a result of early atherogenesis.

Ethnic differences in vascular structure and function: AIx and PWV

Arterial stiffness as measured by PWV is another measure of arterial structure. Although it has been regarded as influenced by vascular risk factors and atherosclerotic change in the arterial wall, it is becoming evident that PWV is largely independent of vascular risk factors other than age and blood pressure. It is likely that it depends on properties of the elastin and collagen in the extracellular material of the arterial wall (London, 2005). AIx is thought to be a composite measure of arterial stiffness and pressure wave reflection, being influenced by vascular smooth muscle tone as well as arterial stiffness. Previous studies in older subjects than in my study have shown either similar (Rezai, 2011) or increased PWV in black African/African-Caribbean subjects compared to white subjects (Chaturvedi, 2003). In the present study, however, we observed no significant difference in PWV or AIx between young black and white subjects. It is possible that higher values of PWV in black subjects in the study by Chaturvedi et al were due to higher blood pressures sustained over many years since, although differences persisted after adjustment for blood pressure at the time of the study, they were not adjusted for long-term blood pressures likely to determine structural adaptation of the arterial wall.

Relationship of FMD to plasma renin activity

The relationship between vascular outcome measures and subject characteristics in this study shows a novel finding, that FMD is strongly and independently associated with PRA irrespective of ethnicity. This finding could help reconcile a number of apparently contradictory findings with respect to endothelium-dependent vasomotor function in hypertensive states whereby endothelial function has been variously reported as preserved or impaired. Renin status has not been reported in such studies and endothelial function may be depressed in low-renin hypertensive states but not necessarily in other hypertensive states. That FMD relates more strongly to renin status than to ethnicity suggests that the varying results of studies on ethnic differences in FMD may be explained by varying renin status of the different ethnic groups. In future studies of FMD and ethnicity, the role of renin needs to be explored.

This cross sectional study does not allow us to make any conclusions regarding causality of the relation between FMD and PRA. It is possible that sodium retention leads to an impairment of availability of endothelium-derived NO or alternatively that NO influences sodium retention. These possibilities will be examined through modulation of salt intake and Chapter 6 and inhibition of NO synthesis in Chapter 7.

It is also important to note that the conclusions can only be generalized to subjects with similar characteristics to those in the study population. Our study was limited to asymptomatic subjects with no known medical history and on no regular medication (i.e. typical of asymptomatic subjects in the general population). Inevitably, given the high prevalence of hypertension in the general population, a minority of subjects may have had undiagnosed hypertension and in future studies it will be important to determine to what extent the presence or absence of hypertension may influence the findings. Many subjects were overweight and again further studies will be required to determine if the present findings vary according to presence or absence of overweight/obesity.

6. SALT LOADING STUDY

6.1 Introduction

The cross sectional study presented in Chapter 5 demonstrated a significant independent positive correlation between FMD and PRA. Salt intake and/or sodium retention is thought to be one of the main mechanisms influencing vascular smooth muscle tone. A trans-membrane ion channel, $\text{Na}^+ - \text{Ca}^+$, is thought to play a major role of regulating the latter in these salt sensitive (low renin) subjects. This channel functions in two states, dependent on its polarity, and these are as $\text{Na}^+ - \text{Ca}^+$ influx (NCX1) and efflux channels. A high level of cytosolic calcium causes smooth muscle contraction and vascular constriction – a key factor in the genesis of hypertension. With the use selective inhibitors for NCX1, such as SEA4000, this channel has been found to play a role in the development of hypertension (Blaustein, 2009).

Subjects with low renin, thought to retain sodium through renal tubular mechanisms as discussed in Chapter 1, are described as “salt sensitive”. This relates to the influence of salt intake on increasing blood pressure when compared with those with higher renin. This has been attributed to the influence of sodium on the peripheral arteriolar tone and hence systemic peripheral resistance. The extent, to which similar mechanisms could influence endothelial function, in particular NO bioavailability, is unclear. However, it has been suggested that a high salt intake is associated with increased oxidative stress

with resultant inactivation of NO by free radicals such as superoxide anion (Kitiyakara, 2003).

The purpose of the study described in this chapter was to examine whether modulation of salt intake, and hence PRA, influences FMD as a measure of NO-dependent endothelial function. Because of the greater salt sensitivity with respect to blood pressure of subjects with low renin, we selected subjects with low PRA and examined effects of a low and high salt intake in a crossover study design. We also examined the influence of modulating salt intake on oxidative stress as measured by urinary $F_{2\alpha}$ -isoprostanes (Roberts II, 2000).

6.2 Aims

- To examine the acute effects of salt loading on vascular function in a low renin salt sensitive cohort.
- To determine the effects of acute salt loading on oxidative stress in a low renin salt sensitive cohort.

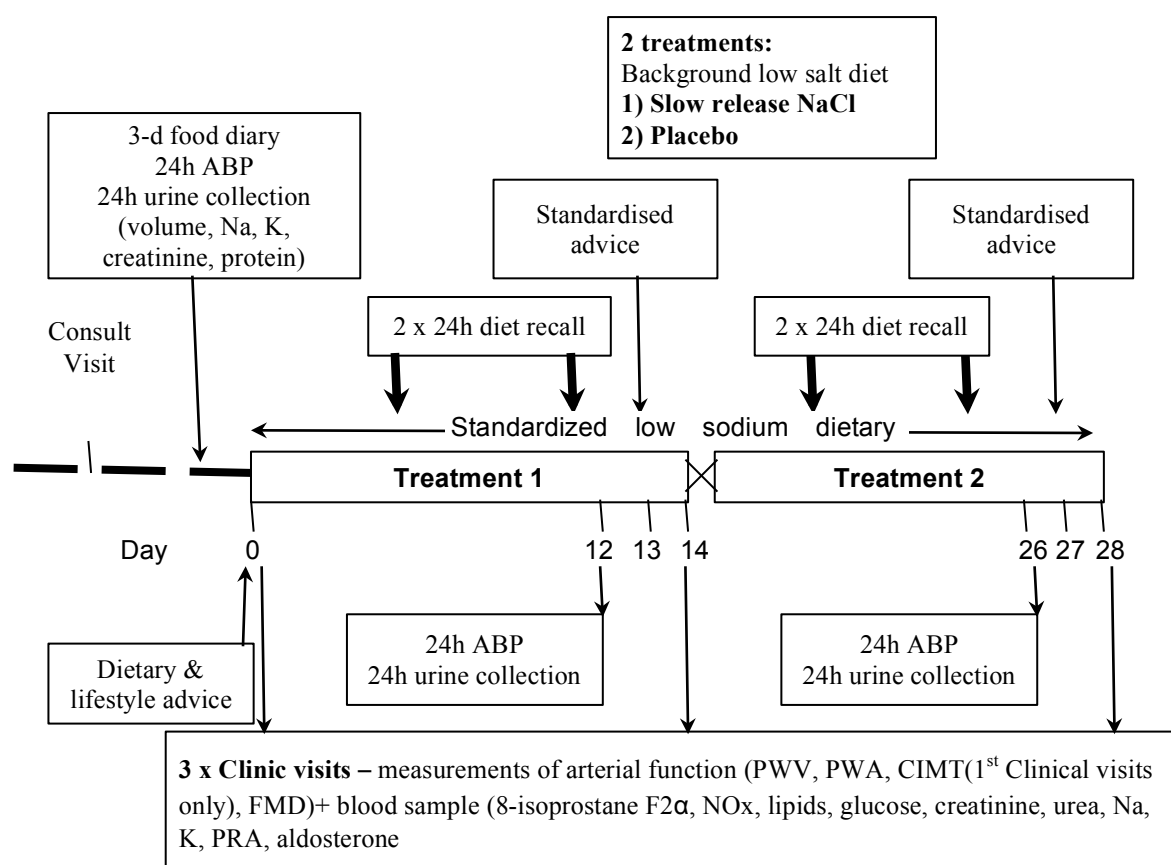
6.3 Recruitment and Participant Characteristics

Twenty-one “low renin” (11 men, 10 women) of the participants who took part in the cross-sectional study described in Chapter 5 were enrolled in the study. Inclusion criteria were identical to the cross-sectional study but additionally included a baseline PRA < 0.3 ng/ml/hr as measured in this previous study. The value of < 0.3 ng/ml/hr was chosen as a threshold as used by other investigators (He, 2002; Duffy, 2005) and it corresponded approximately to the 10th percentile in the previous study (for subjects of all ethnicities). Because in the previous study, FMD was found to associate with PRA but not with ethnicity, PRA was the inclusion criteria rather than ethnicity and both black and white subjects were recruited. Subjects were consented separately for this arm of the study after full disclosure of information both in a telephone interview and after receiving a participant information sheet. Subjects further underwent a dietary consultation visit during which their regular diet was reviewed in consultation with a dietician and they were counseled on the necessary dietary changes required for the study. In addition, we measured oxidative stress by Liquid Chromatography – Mass Spectroscopy (LC-MS) for urinary isoprostanes. Urinary isoprostanes have been used in previous studies as a marker of oxidative stress (Montuschi, 2004).

6.4 Methods

Following the initial dietary consultation, subjects completed a dietary questionnaire and 3 day salt diaries to ascertain the usual sodium intake as determined by “sodium points”. Participants were then given dietary advice to reduce their sodium content to less than 100 points (2.0g of NaCl equivalent) per day. Adherence to this low salt diet was checked through follow-up phone calls made on at least 4 occasions throughout the study period and by 24-hour collections of urine. Twenty-four hour ambulatory blood pressure monitoring and a 24-hour urine collection were arranged on the day before the start of the study. Participants then returned between 08:00hr and 08:30hr after an overnight fast. Venesection was performed for the measurement of serum lipids, sodium, potassium, urea and creatinine. Vascular measurements for pulse wave analysis (PWA), pulse wave velocity (PWV) and flow mediated dilation (FMD) were recorded as for the cross sectional study. Subjects then entered a randomized 2-phase cross-over study design as shown in **Figure 16** where they received 200 mmol of NaCl (as 20 x 10 mmol NaCl tablets) in one treatment phase and matching placebo tablets in the other phase, each phase for 14 days. Ambulatory blood pressure was measured and a 24-hour urine collection performed on day 12. On day 14, subjects returned again at 08:00hr and underwent venesection for serum electrolytes, plasma glucose, PRA and plasma aldosterone and measurement of FMD, PWA and PWV. Blood and urine biochemistry including PRA and aldosterone were measured in a standardized accredited laboratory as described in Chapter 4.

Figure 14: Flowchart of REVERED Salt Loading Study



6.5 Results

The results of the salt intervention study are shown in **Table 6**. All 21 participants enrolled completed the study and these included 16 black and 5 white participants. **Table 6** shows the baseline characteristics of the subjects. Despite subjects being selected on the basis of $\text{PRA} < 0.3 \text{ ng.ml}^{-1}\text{hr}^{-1}$ in the previous study, the median PRA in the present study on the high salt diet, was $0.3 \text{ ng.ml}^{-1}\text{hr}^{-1}$.

Table 6: Baseline characteristics of volunteers (n=21) in salt loading study

Characteristic		Mean	SD
Age	(years)	29	10
BMI	(Kg/m ²)	26.1	3.8
Seated BP: Sys	(mmHg)	122	14
	Dias (mmHg)	73	11
	HR (bpm)	59	7
ABPM: Sys	(mmHg)	127	13
	Dias (mmHg)	75	9
Fasting glucose	(mmol/L)	4.7	0.5
Serum Na	(mmol/L)	143	2
Serum K	(mmol/L)	4.3	0.3
Creatinine	(μmol/L)	74	10
Lg PRA	(log ng/ml/hr)	-0.04	0.18
Urinary Na:Cr		9.0	3.4
24hr U Na	(mmol/d)	138	68

BMI, body mass index; BP, blood pressure; Sys, systolic; Dias, diastolic; HR, heart rate; ABPM, ambulatory blood pressure monitoring; Na, sodium; K, potassium; Lg PRA, log (base 10) plasma renin activity; Urinary Na:CR, urinary sodium to creatinine ratio; 24hr U Na, 24 hour urinary sodium.

Table 7: Blood pressure, vascular function and biochemistry on low (placebo) and high (NaCl) sodium diets

Measure	Placebo		Salt		P value
	Mean	SD	Mean	SD	
ABPM Systolic (mmHg)	124	12	133	16	0.023
ABPM Diastolic (mmHg)	75	9	82	13	0.106
PWV (ms⁻¹)	10.0	2.1	10.6	2.3	0.175
AIx (%)	53.9	22.0	61.4	17.8	0.286
FMD (%)	5.6	4.0	6.3	4.1	0.613
U Na:Creatinine	6.8	3.1	18.8	7.5	0.010
PRA (ngml⁻¹hr⁻¹) *	0.9	[0.6, 1.4]	0.3	[0.2, 0.95]	<0.001
Urinary 2,3 Dinor 8-IsoF_{2α} (nM) / Creatinine (mM)	6.387	2.976	6.082	3.204	0.556
Urinary 8-Iso-PGF_{2α} (nM) / Creatinine (mM)	0.687	0.341	0.673	0.423	0.657

ABPM, ambulatory blood pressure monitoring; PWV, pulse wave velocity; AIx, augmentation index; FMD, flow mediated dilation; U Na:Creatinine, urinary sodium to creatinine ratio; PRA, plasma renin activity.

* Values are medians and interquartile range.

Table 7 shows that there was an approximately 3-fold increase in urinary sodium excretion, as assessed by the 24-hour urinary sodium/creatinine excretion after salt loading on the background of the low salt diet. Salt loading led to a reduction in PRA and to an increase in 24-hour ambulatory systolic blood pressure (124 mmHg at baseline on the low salt diet vs. 133 mmHg after salt loading, $P = 0.023$). However, there was no significant difference in FMD, AIx or PWV after salt loading. There were no significant interactions between change in FMD after salt loading and ethnicity.

6.6 Conclusion

The results suggest that short-term oral salt loading reduces PRA. There was also a predicted increase in the systolic blood pressure response on 24 hour monitoring. There was no significant difference in measures of arterial stiffness or flow-mediated dilatation. This suggests short-term salt loading may cause volume dependent changes without alteration in either endothelial function or vascular stiffness.

6.7 Discussion

This sub-study examined vascular and biochemical parameter changes after short-term salt loading. A clear difference between sodium excretion was demonstrated in the placebo versus salt phases of the study. There was a concomitant fall in PRA on salt loading as demonstrated in previous studies for both oral and intravenous salt loading and there was a difference of 9 mmHg in ambulatory systolic BP between the high and low salt phases of the study. In the 2011 Cochrane review by Graudal *et al* looking at the effects of oral salt on blood pressure in 62 trials, they noted a fall of 1.27 mmHg in normotensive Caucasians on salt restricted diets (Graudal, 2012). Lack of a change in diastolic BP is consistent with observations in the Graudal review with meta-analysis showing no significant difference in DBP. This review further supported a greater fall in SBP (-4.02 mmHg [95%CI - 7.37, -0.68 mmHg]) in black compared to white subjects in a sub meta- analysis including 6 trials with black participants. The relatively large change in SBP in our study might reflect the high proportion of black versus white subjects enrolled.

This study did not demonstrate a significant change in FMD on acute salt loading or restriction. Dickinson *et al* 2011 showed in a study of a high salt meal (HSM) 65 mmol Na compared to low salt meal (LSM) 5 mmol Na, a significant difference in FMD between the two interventions (3.39% +/-2.44% after HSM versus 6.05% +/- 3.21% after LSM) as early as 30 minutes after the intervention (Dickinson, 2011). The renin status in this study, however, was not characterized. The lack of significance shown in our study might be explained

by both a type 2 error and the renin status of the participants in our study with a relatively high percentage of low renin subjects. The low renin state might reflect chronic changes to the endothelium either directly from Na or another mediator such as oxidative stress. These would likely not be reversible on acute sodium concentration changes in the vascular compartments.

Our study had a number of limitations: 1) the small sample size which makes it difficult to exclude a type 2 error. 2) despite subjects being selected on the basis of $\text{PRA} < 0.3 \text{ ng.ml}^{-1}\text{hr}^{-1}$, the median PRA in the present study on the high salt diet, was $0.3 \text{ ng.ml}^{-1}\text{hr}^{-1}$. This may represent a regression to the mean and/or be a reflection of the inherent variability of PRA. Subjects of varying ethnicities were studied and this could have given rise to some confounding (although results were not influenced by adjustment for ethnicity), if there are ethnic differences in response to salt loading unrelated to PRA. Thus a future study should study a larger group of low renin subjects that would allow separate analysis in different ethnic groups.

7. Nitric Oxide Inhibition Sub-study

The effects of acute inhibition of nitric oxide synthesis on urinary sodium excretion

7.1 Introduction

A tendency to sodium retention with suppressed plasma renin activity (PRA) is thought to be more common in subjects of African ancestry than in white subjects and may account for a greater prevalence and severity of hypertension in black compared to white subjects. The underlying cause of the “low renin” phenotype is unknown, but, as described in Chapter 1, could be due to increased renal tubular reabsorption of sodium. NO is known to influence renal tubular absorption of sodium causing reduced reabsorption in the cortical collecting duct and therefore a potential natriuresis (Stoos, 1995). NO synthesised from eNOS in the endothelium of peri-tubular renal vasculature and/or NO released from other sources such as neuronal NOS (nNOS) could, therefore, influence sodium homeostasis. Reduced NO availability could therefore potentially explain reduced endothelial vasomotor function (e.g. reduced FMD), increased sodium reabsorption and low renin status, features that have been reported to be more common in subjects of African ethnicity. In this chapter I examined the influence of acute inhibition of NOS using N^G-monomethyl-L-arginine (L-NMMA), a non-specific inhibitor of NOS. I hypothesized that, if NO availability is responsible for sodium homeostasis and reduced NO availability contributes to a low renin state that a) acute NOS inhibition would reduce sodium excretion and b) effects of L-NMMA to reduce sodium excretion would be reduced in subjects with low PRA.

7.2 Methods

Subjects were a sub-group (n=40) of those studied in Chapter 5. Subject characteristics are shown in *Table 8*. Subjects attended the vascular laboratory in the morning at approximately 08:00 after an overnight fast. Venous cannulae were inserted for drug administration and blood sampling and subjects commenced an oral water load of 200ml per hour. After 30 min lying semi-supine, hourly urine collections were commenced. After 2 hours, subjects received an intra-venous infusion of the NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA, 3mg/Kg over 5 min), following which hourly urine collections were continued for a further 2 hours. Blood pressure was monitored using an oscillometric monitor (Omron 705CP) before and at 5 minute intervals for 20 minutes and then, 1 hour and 2 hours after the infusion of L-NMMA. Six subjects attended on a separate occasion and received saline placebo instead of L-NMMA. Urinary sodium (U_{Na}), plasma sodium (P_{Na}) urinary creatinine (U_{Cr}) and plasma creatinine (P_{Cr}) concentrations were measured in an accredited laboratory. To take into account variation in sodium intake, fractional urinary sodium excretion (FE_{Na}) was calculated from urinary and plasma values according to the formula:

$$FE_{Na} = \frac{U_{Na} \times P_{Cr}}{P_{Na} \times U_{Cr}}$$

7.3 Statistical analysis

Values of FE_{Na} at baseline and after infusion of L-NMMA/placebo were taken as the average of those obtained for the 2 hours before and after infusion of L-NMMA/placebo respectively. Values of FE_{Na} after L-NMMA/placebo were compared with baseline values by Student's paired t-test. The relation of the change in FE_{Na} from baseline after L-NMMA to PRA was examined using multiple regression analysis using log-transformed values of PRA as a continuous variable and, using ANOVA, across 3 categories of PRA: < 0.5 , $0.5 - 1.0$ and > 1.0 ng/mL/h, corresponding to approximate tertiles of the distribution of PRA. In both analyses, baseline values of FE_{Na} were included as a covariate and an additional model was examined in which age, sex and ethnicity were also included.

7.4 Results

Table 8: Baseline characteristics of participants (n=40) in the LNMMA sub study.

Measure		Mean (SD)
Age	(Years)	30(10)
BMI	(Kg m ⁻²)	26.4(4.7)
Waist: Hip		0.81(0.07)
Seated BP	(mmHg)	
Systolic		123(13)
Diastolic		77(10)
Total Chol.	(mmol/L)	4.2(1.0)
LDL	(mmol/L)	2.41(0.78)
HDL	(mmol/L)	1.54(0.39)
TAG	(mmol/L)	0.73(0.39)
Glucose	(mmol/L)	4.5(0.6)
Serum Na	(mmol/L)	140(2.0)
Serum K	(mmol/L)	4.1(0.2)
Creatinine	(mmol/L)	80(15)
PRA	(ng ml ⁻¹ hr ⁻¹) ¹	0.9 (0.41.2)
Urine Na: Cr		11.5(5.0)

BMI, body mass index; BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; TAG, triacylglycerol; Na, sodium; K, potassium; PRA, plasma renin activity; Urine Na:Cr, urine sodium to creatinine ratio.

¹ Values are median (interquartile range)

Haemodynamic effects of LNMMA

Administration of a saline vehicle was without significant effect on heart rate or blood pressure. After L-NMMA there was a small but significant increase in blood pressure from $122\pm2.6/74\pm1.8$ mmHg at baseline to $126\pm2.5/77\pm2.0$ mmHg 15 min after L-NMMA (each $P<0.001$) and decrease in heart rate from 61 ± 1.4 to 56 ± 1.1 bpm ($P<0.0001$).

Effects of L-NMMA on renal sodium excretion and relation to PRA

FE_{Na} did not change significantly after saline placebo (0.80 ± 0.09 vs $0.85\pm0.10\%$ at baseline and after placebo respectively). However, after L-NMMA, FE_{Na} decreased from 0.64 ± 0.03 to $0.57\pm0.03\%$ ($P<0.0001$), corresponding to a 12% reduction in FE_{Na} . The reduction in FE_{Na} by L-NMMA was significantly positively correlated with PRA (**Figure 15**) when adjusted for baseline values of FE_{Na} alone ($P<0.01$) or baseline values of FE_{Na} , age, sex and ethnicity ($P<0.05$). Thus, in subjects with $PRA > 1.0$ ng/mL/h, L-NMMA reduced FE_{Na} by $29.6\pm5.5\%$ but in subjects with $PRA < 0.5$ ng/mL/h, L-NMMA reduced FE_{Na} by only $3.4\pm7.0\%$ ($P<0.01$).

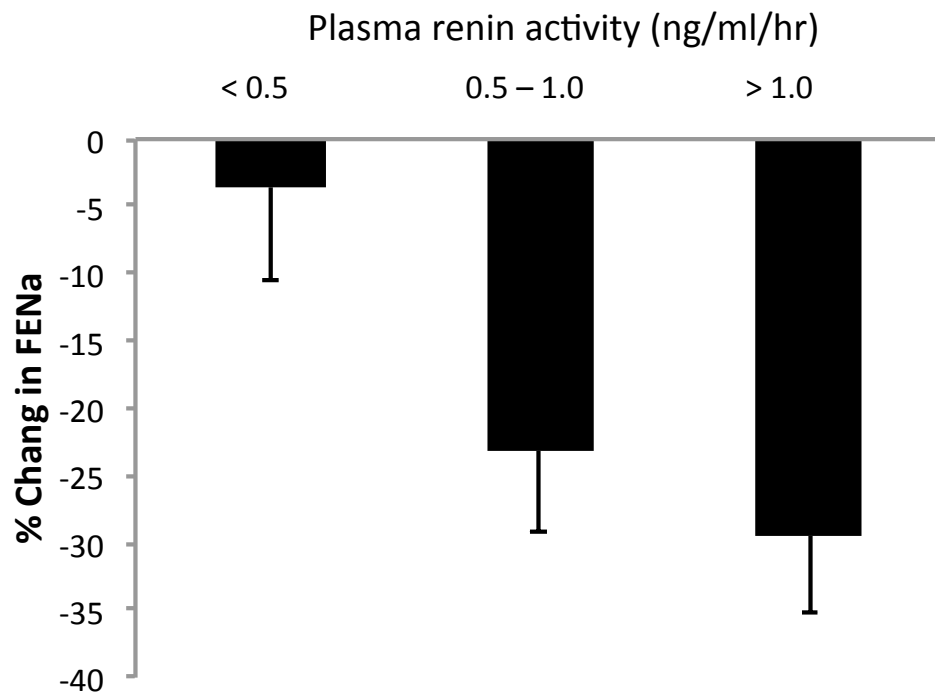


Figure 15: Change in fractional excretion of sodium (FENa) after NO synthase inhibition according to baseline plasma renin activity

7.5 Conclusion

In conclusion, this study suggests that, in asymptomatic normotensive subjects, renal sodium excretion is, in part, regulated by endothelium-derived or other peritubular source of NO and that reduced NO availability may underlie sodium retention and low PRA. Interventions to increase endogenous NO availability and /or NO donors should be explored as treatments to prevent or treat hypertension associated with the low renin phenotype.

7.6 Discussion

Recent findings pointing to a role of NO in renal tubular sodium excretion, raise the possibility that NO availability might be a determinant of sodium excretion and hence renin status. To examine this possibility I assessed the response of NO synthase inhibition on urinary sodium excretion, reasoning that reduced availability of NO would be associated with reduced response to NO synthase inhibition. The NO synthase inhibitor L-NMMA markedly reduced urinary sodium excretion. This confirms earlier observations in a small cohort of asymptomatic subjects that tonic release of NO from the renal vasculature or elsewhere is involved in regulation of sodium excretion (Bech, 1998). Importantly, the effect of L-NMMA to inhibit sodium excretion was reduced in subjects with low PRA. Taken together, the effect of L-NMMA to inhibit sodium excretion and impaired anti-natriuretic effects of NO synthase inhibition in subjects with low PRA suggest that vascular NO availability is a major determinant of sodium balance and renin status with low renin status relating to reduced NO availability. Results of the study appear to contradict findings of Davidson et al. who reported that, after sodium depletion with furosemide, L-NMMA infusion caused an increase in PRA (Davidson, 1999). However, pre-treatment with furosemide makes this study difficult to interpret because subjects may not have been in sodium balance. Further work to examine the anti-natriuretic effect of L-NMMA during low and high salt diets will be required to understand these apparent contradictory findings.

A potential role of NO in regulation of sodium balance and renin status has important implications. In the presence of risk factors such as obesity, insulin resistance, dyslipaemia and diabetes all of which reduce NO availability, it provides an additional mechanism underlying the association of these conditions with hypertension. It suggests that treatments that increase endogenous NO availability and/or NO donors may be useful in reducing sodium retention. In this regard it is notable that NO donors are particularly effective in increasing PRA, with effects similar to diuretics and, when used in heart failure, are thought to offer greater clinical benefit when used in black compared to white subjects (Taylor, 2004). Future studies should explore whether other co-morbidities that are associated with low renin are also associated with sodium retention and low PRA and whether interventions that modulate NO availability will influence PRA. One possibility would be to use methodology described by Forte et al (Forte, 1998) to measure whole body NO production as well as indirect measures of endothelium derived NO production, such as FMD, to determine whether interventions such as weight loss or “statin” treatment will increase NO production and reduce sodium retention.

A decline of PRA with age is well recognized (Tsumoda, 1986). Various mechanisms have been hypothesized to account for this such as age-related alterations of the kidney (decreased number of functional nephrons) that could influence the active to inactive plasma renin ratio (Weidmann, 1975), diminished synthesis of angiotensin by the liver (Leubel, 2008) and (in women) decreased levels of estradiol and progesterone (hormones that both stimulate secretion of renin (Sealey, 1994)). A role of nitric oxide in determining PRA

also provides a potential explanation for the association of ageing (a major determinant of NO availability (Di Massimo, 2006)) with sodium retention, diminished PRA and higher blood pressure.

These results also have implications for the interpretation of studies on endothelial function where an NO mediated vasodilator response such as FMD is used as a measure of generalized endothelial cell function. It is likely that, particularly in multi-ethnic cohorts and in hypertension studies, FMD relates to renin status (which is rarely measured). In subjects with essential hypertension, there are divergent findings with regard to measures of endothelial function: some studies report impaired and others preserved endothelial function. It is possible that these findings are explained by differing renin status of hypertensive groups in the different studies. Whilst sodium retention and low renin status are associated with greater prevalence and severity of hypertension and stroke it does not necessarily associate with increased incidence of coronary artery disease. Furthermore, recent studies indicate that endothelium-derived NO availability, as measured by FMD, is not independently related to cardiovascular outcomes and it is possible that NO availability is a more important determinant of sodium balance than of atherogenesis or progression of atherosclerotic disease.

This study is subject to a number of important limitations. First we studied a cohort of apparently asymptomatic subjects to examine the potential role of NO in influencing sodium balance in normal physiology. The role of NO is likely to

differ in subjects with neurohormonal or renal hypertension/disease such as primary aldosteronism where aldosterone may be the dominant determinant of sodium balance. Finally, the NO synthase inhibitor we used is non-specific, inhibiting all NOS isoforms including eNOS and neuronal NOS. Thus we cannot be certain as to which isoform is involved in renal effects on sodium balance.

8. THESIS DISCUSSION

8.1 Overview

As noted in Chapter 1, there are well-established differences in CVD risk and events between black and white ethnic groups and these differences account for significant health disparities. Both traditional and non-traditional risk factors are thought to play a role (Muntner, 2005). One major risk factor appears to be the relatively higher prevalence of hypertension in black compared to white subjects which may contribute to the higher mortality and morbidity observed in black compared to white groups; especially in relation to haemorrhagic stroke. It has been mentioned earlier that this difference is likely to diverge further if interventions targeted at a regional, national and international level are not implemented. A subtype of the hypertension epiphenomenon, salt sensitive hypertension, has been clearly described as especially sensitive to salt (NaCl) intake. Renin, a hormone involved in sodium regulation, has been shown by a number of investigators to be lower in black compared to white subjects in both normotensive and hypertensive subjects. This was confirmed in normotensive subjects in Chapter 5 where a significant difference in prevalence of “low” renin” as defined by $\text{PRA} < 0.3 \text{ ngml}^{-1}\text{hr}^{-1}$ (black: 54% vs. 6% in white) was observed. Although less extensively studied than renin status and salt-sensitive hypertension, another recognized difference between black and white ethnic groups is a difference in vascular function, particularly endothelium-derived, NO-dependent vascular function (Bild, 2002). Since NO is recognized to influence renal tubular sodium absorption and sodium excretion availability of

endothelium-derived NO from eNOS, or another peritubular source of NO could represent a common factor linking sodium homeostasis with vascular function and structure (Romero, 1992).

In this thesis, I have looked for differences between black and white ethnic groups in blood pressure, measures of vascular structure (CIMT and arterial stiffness as measured by PWV), vascular function (FMD and GTND) and sodium homeostasis; examined blood pressure and vascular responses to modulation of dietary salt; and effects of inhibition of NO synthase inhibition on renal sodium excretion in a white/black ethnic groups in South East London. The hypothesis that there is a causal relationship between NO availability, renal sodium handling and vascular dysfunction was investigated by relating effects of NO synthase inhibition and NO mediated vasodilation as measured by FMD to renin status.

8.2 Sodium homeostasis and ethnicity

Renin status and 24-hour urinary sodium were measured in Chapter 5. Previous investigators have used 24 hour-urine collections as a measure of usual sodium intake. Although some investigators have reported higher levels of dietary salt intake in black compared to white ethnic groups (Cappuccio, 1997), we observed similar 24 urinary sodium excretion and therefore, by extrapolation, sodium intake. However, we studied a relatively homogenous socioeconomic group of subjects recruited mainly from higher educational establishments. Differences in sodium intake in other studies may have been due, at least in part, to differing socioeconomic status and different diets. PRA was not correlated with 24-hour sodium excretion and differences in 24-hour sodium excretion did not account for ethnic differences in PRA, which remained significantly lower in black compared to white subjects when adjusted for 24-hour sodium excretion. Thus lower PRA in black compared to white subjects in our study is likely due to renal sodium handling rather than dietary intake. This is consistent with previous studies where no correlation between PRA and sodium intake has been observed (Sagnella, 2001).

8.3 Vascular structure and function in relation to ethnicity

In Chapter 5, I looked for differences in blood pressure, FMD, GTND, CIMT and carotid-femoral PWV in relation to self defined ethnicity. FMD is of particular interest since it is mediated almost exclusively by eNOS. Although FMD tended to be lower in black compared to white subjects, the difference did not reach statistical significance. However, FMD was significantly correlated to PRA. Whilst this could have occurred as a result of confounding, the relationship was not affected by adjustment for other risk factors (including ethnicity) known to be associated with FMD. This relationship between FMD and PRA, therefore, suggests a relation between sodium hemostasis and NO-dependent vascular function. Vasodilation to the NO donor GTN was similar in the two ethnic groups and was not significantly correlated with PRA, suggesting that any relation between sodium homeostasis and vascular function arises as a result of the availability of endothelium-derived (or other endogenous source of NO) rather than the response to NO. CIMT, a measure of vascular structure thought to be a sub-clinical measure of atherosclerosis and/or a measure of vascular smooth muscle hypertrophy was significantly greater in black compared to white subjects. Differences seen in CIMT were not explained by PRA, BP or FMD. This suggests structural changes in the arterial wall are driven, at least in part, by factors other than sodium retention, BP and NO availability. However, we observed no correlation with other risk factors that could explain the difference in CIMT between the ethnic groups. This is consistent with previous studies (Fulsom, 2008) that have shown no correlation between traditional risk factors, such as glucose and lipid sub-fractions, and

CIMT in African subjects. By contrast to CIMT, PWV was not significantly different between black and white subjects. PWV is thought to be driven mainly by blood pressure and this is consistent with little difference in blood pressure between the groups (Hansen, 2006).

8.4 Relation between vascular function and sodium homeostasis

The key finding of this thesis that FMD is independently related to PRA could have arisen because sodium retention impairs NO availability. Sodium retention could for example increase oxidative stress and impair eNOS derived NO availability or there could be a direct interaction between renin and eNOS/NO. However, an alternative explanation is that NO availability determines sodium homeostasis. A number of studies in isolated cells and in animal models suggest that NO inhibits renal tubular absorption (Davidson, 1999). It might also be suggested that another distinct factor might regulate both NO bioavailability and sodium excretion. Endothelial dysfunction has been shown in previous studies (Di Massimo, 2006)) to be associated with chronic inflammation and thus inflammatory mediators may represent a possible candidate. To distinguish between these possibilities I examined the influence of changing salt intake on FMD and the influence of acute inhibition of NOS on renal sodium excretion.

8.5 Modulation of dietary Salt intake

In Chapter 6, I examined effects of modulation of dietary salt intake on BP, renin status and FMD. Compared to salt restriction, salt loading decreased renin and increased BP but had no significant effect on FMD. This contrast with results in a similar dietary sodium intervention study Dickenson *et al.* (Dickinson, 2011) These investigators randomized forty-one subjects to a low (50 mmol/day Na) vs. a “usual diet” (150 mmol/day Na). They demonstrated significant changes in both FMD (4.89 +/- 2.42%) in the low salt diet vs. (3.37 +/- 2.1%) in the usual salt group (P=0.02). In this study, participant’s renin status was not characterized. However, the geographical location of this study means that it was likely that the participant’s PRA was higher than in our study. Although a lack of effect on FMD in my study could be due to a type 2 error, the difference between my findings and those of Dickenson et al may be due to lack of effect of additional sodium loading in a state where there is already sodium retention. In this case, the finding of a lack of effect of modulation of sodium intake on FMD, suggests that, if there is a link between NO availability and sodium homeostasis, it is unlikely to be due to sodium retention influencing NO availability, in subjects with low renin as in my study.

8.6 Influence of NO synthase inhibition on urinary sodium excretion

To test the hypothesis that NO availability influences sodium homeostasis directly, thus explaining the link between FMD and renin status, I examined the influence of NO synthase inhibition on urinary sodium excretion in Chapter 7. Acute eNOS inhibition with L-NMMA reduced urinary sodium excretion and did so in a manner that was greater in subjects with higher compared to lower renin. This suggests that NO availability influences sodium homeostasis through renal sodium handling. Relative lack of effect of NOS inhibition in subjects with lower renin may be due to low NO availability due to low basal NO synthesis in those subjects and /or to increased NO destruction and lead to increased risk of vascular disease associated with reduced NO availability.

8.7 Synthesis and interpretation of experimental findings

In summary, the main experimental results from Chapters 5, 6 and 7 are that:

- FMD is independently correlated with renin status.
- Salt modulation does not influence FMD in subjects with low renin.
- Acute NOS inhibition impairs sodium excretion to a degree that relates to PRA.

These experimental findings support the hypothesis that:

- NO availability is a determinant of sodium homeostasis.
- Reduced NO availability may contribute to the tendency for reduced endothelium-dependent vascular function and sodium retention in subjects of black African/ black Caribbean ethnicity.

8.8 Implications – Clinical Pharmacology and Therapeutics

Current management of hypertension in most major guidelines, such as British Hypertension Society (BHS) and the National Institute for Clinical Excellence (NICE), advocate both lifestyle and pharmacological treatment.

One lifestyle change advised is dietary interventions to reduce sodium consumption. The direct link between dietary salt intakes and hypertension has been demonstrated in prospective and retrospective studies. This relationship may be even more critical in low renin states. In the Dietary Approaches to Stop Hypertension – Salt diet (DASH- Salt) a sub study of DASH, participants were restricted to 3g, 2.4g and 1.5g of sodium for 30 days. The lowest salt diet produced the greatest reduction in BP of approximately 8.9/4.5 mmHg.

Pharmacological management of hypertension has principally been divided into drugs contributing to natriuresis and those causing arteriolar relaxation. The former has been cardinal in the management of low renin (salt sensitive) hypertension and includes both thiazide diuretics and aldosterone antagonists. Considering the arguments presented in this thesis, with the possible role of NO availability in sodium handling, drugs increasing NO availability may also play a role in hypertension management in this group.

The HMG-Co A reductase inhibitors, or “statins”, are thought to have pleiotropic effects in increasing NO bioavailability. Statins are thought to have a modest effect in reducing blood pressure but could have a greater effect in

subjects with low renin in whom they might reduce sodium retention. NO donors like glyceryl trinitrate might also be more efficacious in patients with low renin and it is notable that they are thought to provide more benefit to black compared to white patients with heart failure (Taylor, 2004).

Diminished availability of NO in association with risk factors traditional and novel could lead to increased risk not only through pro-atherogenic effects within the vascular endothelium but through sodium retention; this might explain why renin goes down with age.

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APPENDICES

APPENDIX 1 – ETHICS APPROVAL FORM

St Thomas' Hospital Research Ethics Committee

South London REC Office 3
Ethics Committee Office
Governors' Hall Suite,
Ground Floor South Wing
St Thomas' Hospital
London
SE1 7EH

Telephone: 0207 188 2257
Facsimile: 0207 188 2258

12 February 2008

Professor Philip Chowienczyk
Professor of Cardiovascular Pharmacology
Dept of Clinical Pharmacology
South Wing, Block 5
St Thomas' Hospital

Dear Professor Chowienczyk

Full title of study: Ethnic differences in blood pressure and vascular function
REC reference number: 07/H0802/135

Thank you for your letter of 08 February 2008, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA). There is no requirement for [other] Local Research Ethics Committees to be informed or for site-specific assessment to be carried out at each site.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Application		06 November 2007
Investigator CV		
Protocol	1	06 November 2007
Questionnaire: Diary Questionnaire of Daily Life		
Questionnaire: TICS-LE	1.0	
Questionnaire: HAD Scale		
Questionnaire: Perceived Stress Scale		
Participant Information Sheet: L-NMMA study	1.1	08 February 2008
Participant Information Sheet: Blood pressure and ultrasound	1.1	08 February 2008
Participant Consent Form	1.1	08 February 2008
Response to Request for Further Information		08 February 2008

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from <http://www.rdforum.nhs.uk/rdform.htm>.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

Here you will find links to the following

- a) Providing feedback. You are invited to give your view of the service that you have received from the National Research Ethics Service on the application procedure. If you wish to make your views known please use the feedback form available on the website.
- b) Progress Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
- c) Safety Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
- d) Amendments. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
- e) End of Study/Project. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nationalres.org.uk .

07/H0802/135	Please quote this number on all correspondence
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With the Committee's best wishes for the success of this project

Yours sincerely

Dr Adrian Hopper
Chair

Email: stella.hirsch@gstt.sthames.nhs.uk

Enclosures: Standard approval conditions

Copy to: Mr Keith Brennan,
R & D Office, Guy's & St Thomas' NHS Foundation Trust

KCL

APPENDIX 2 – PATIENT INFORMATION SHEET 1

INFORMATION FOR AND CONSENT OF PARTICIPANTS

**RenoVascular function Ethnicity Renin and
Endothelial Dysfunction**

Department of Clinical Pharmacology

St Thomas' Hospital London

CONFIDENTIAL

Ethnic Differences in Renovascular Function
Participant Information Sheet: blood pressure and ultrasound study
07/H0802/135
(version 1.1 08-02-08)

1. Study Title

Ethnic differences in renovascular function

2. Invitation Paragraph

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

3. What is the purpose of the study?

Black African and Afro-Caribbean subjects have, on average, a greater risk of a stroke than white subjects living in South London. This may be because black subjects have higher blood pressure and arteries that relax less readily in response to stress than white subjects. These differences may, in turn, be due to greater retention of sodium by the kidney in black compared to white subjects. The purpose of this study is to compare blood pressure and arterial function in relation to kidney function in black and white subjects. This will help us to find out why black subjects are more at risk of stroke and will help us to prevent stroke in both black and white subjects.

4. Why have I been chosen?

You are being invited to take part in the study because you are a healthy volunteer at low risk of cardiovascular disease.

5. Do I have to take part?

It is up to you whether or not you want to take part. If you decide to take part you will be given this information sheet to keep and asked to sign a consent form. However, you are still free to withdraw at any time and without giving a reason.

6. What will happen to me if I take part?

If you decide to take part you will be asked to attend the department on one or two separate occasions.

On the first visit (10 minutes) we will complete your consent form and fit you with an automatic blood pressure monitor designed to be worn with a loose sleeved shirt/top. You will also be given a container to collect urine for 24 hours.

You will be asked to return for visit 2(2 hours) to have the blood pressure monitor removed and to return the urine collection. In addition, on the second visit we will ask you to attend in the morning having fasted from midnight. We will take measurements of your height, weight, waist circumference and blood pressure. We will take a blood sample to check your cholesterol and other markers of cardiovascular risk and hormones that regulate sodium balance. We will make some measurements of the health of your arteries by using an ultrasound scanner to look at arteries in your arm and neck. We will repeat the ultrasound measurements on the arm before and after inflating a blood pressure cuff for 5 minutes and after placing a drop of a drug glyceryl trinitrate on your tongue. Inflating the blood pressure cuff causes a feeling of tingling in the arm and is slightly unpleasant. Glyceryl Trinitrate is a standard drug used to relax arteries. It can cause dizziness and headache but the dose we will use is very low and no side effects have been recorded with this low dose. We will also record your pulse in the finger, arm and thigh by using a blood pressure cuff. The visit will take about 2 hours.

Depending on the results from the first visit, you may be asked to participate in a second study if you wish to do this.

7. What do I have to do?

You will need to lie still during the measurements.

8. What are the possible disadvantages and risks of taking part?

- You will have the discomfort of having the blood sample.
- We may discover that you have high blood pressure and/or high cholesterol that requires further investigation/treatment. If so we will ask you to contact your GP to arrange this.
- The ultrasound test might reveal some thickening of the arteries that requires further investigation/treatment. If so we will ask you to contact your GP to arrange this.

9. What are the possible benefits of taking part?

You will have your blood pressure and cholesterol checked.

10. What if new information becomes available?

Sometimes during the course of a research project, new information becomes available. If new information becomes available that has implications for any treatment or investigation that you should receive we will contact yourself and your GP.

11. What if something goes wrong?

If you are harmed by taking part in this research and this is found to be due to someone's negligence, you have grounds for legal action and compensation. In addition if you have any complaints about a member of the study team we would ask you to inform another member of the study staff about your concerns.

12. Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the study will be kept strictly confidential. Any information that leaves the hospital will have your name and address removed. For this reason, on entering the study you will be assigned a unique patient number that will be used together with your sex and date of birth to identify study documents. If you agree, your GP will be informed of your participation in the trial.

13. What will happen to the results of the research study?

The results from the study may be published by a medical journal and/or presented to the regulatory authorities. This will be done confidentially and under no circumstances will your name be disclosed.

14. Who is organising and funding the research?

The study is being organised and funded by the Cardiovascular Division of King's College London and will be performed in the Department of Clinical Pharmacology, St Thomas' Hospital.

15. Who has reviewed the study?

The ethics for this study has been reviewed by St. Thomas' Hospital NHS Research Ethics Committee.

16. Contact for further information

If you have any further questions or queries about any aspect of the study at any time, then please do not hesitate to contact Dr. Kenneth Connell (020 7188 4770 normal working hours). Thank you for considering taking part in the study. If you decide to take part in the study you will be given a copy of the subject information sheet and a signed consent form to keep.

APPENDIX 3 – PATIENT INFORMATION SHEET 2

INFORMATION FOR AND CONSENT OF PARTICIPANTS

RenoVascular function Ethnicity Renin and Endothelial Dysfunction

Department of Clinical Pharmacology

St Thomas' Hospital London

CONFIDENTIAL

Ethnic Differences in Renovascular Function
Participant Information Sheet: blood pressure and ultrasound study
07/H0802/135
(version 1.1 08-02-08)

1. Study Title

Ethnic differences in renovascular function

2. Invitation Paragraph

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

3. What is the purpose of the study?

Black African and Afro-Caribbean subjects have, on average, a greater risk of a stroke than white subjects living in South London. This may be because black subjects have higher blood pressure and arteries that relax less readily in response to stress than white subjects. These differences may, in turn, be due to greater retention of sodium by the kidney in black compared to white subjects. The purpose of this study is to compare blood pressure and arterial function in relation to kidney function in black and white subjects. This will help us to find out why black subjects are more at risk of stroke and will help us to prevent stroke in both black and white subjects.

4. Why have I been chosen?

You are being invited to take part in the study because you are a healthy volunteer at low risk of cardiovascular disease.

5. Do I have to take part?

It is up to you whether or not you want to take part. If you decide to take part you will be given this information sheet to keep and asked to sign a consent form. However, you are still free to withdraw at any time and without giving a reason.

6. What will happen to me if I take part?

If you decide to take part you will be asked to attend the department on four separate occasions. On the first visit you will receive general information about the study as well as a dietary consult. You will be given detailed instructions on how to avoid high salt content foods. In addition, you will be given a 24hr urine collection and blood pressure monitor to be used two days before your next visit a week later. This visit should last approximately 30 minutes.

On visit 2, at the end of the first week, we will make some measurements of the health of your arteries by using an ultrasound scanner to look at arteries in your arm and neck. We will repeat the ultrasound measurements on the arm before and after inflating a blood pressure cuff for 5 minutes and after placing a drop of a drug glyceryl trinitrate on your tongue. Inflating the blood pressure cuff causes a feeling of tingling in the arm and is slightly unpleasant. Glyceryl

Trinitrate is a standard drug used to relax arteries. It can cause dizziness and headache but the dose we will use is very low and no side effects have been recorded with this low dose. We will also record your pulse in the finger, arm and thigh by using a blood pressure cuff. Finally, we will take a blood sample to check your cholesterol and other markers of cardiovascular risk and hormones that regulate sodium balance. This visit will take about 2 hours.

You will then be asked to take either salt tablets or placebo (dummy tabs) **for two weeks each** (5 tabs x 4 times daily) so that you will either be taking a low or high salt diet for that period. We will keep in touch with you by phone to check that you are happy with the diet and to offer further advice. The order in which you take placebo versus salt tablets will be determined on a similar matter to tossing a coin.

At the end of the third and fifth weeks (visits 3 and 4) you will be asked to attend the laboratory to have repeated blood and urine test, blood pressure and ultrasound measurements.

A high salt diet is known to cause high blood pressure but when taken for just two weeks will not cause a long-term rise in your blood pressure (or any other problems)

7. What do I have to do?

You will need to lie still during the measurements.

8. What are the possible disadvantages and risks of taking part?

- You will have the discomfort of having the blood sample.
- We may discover that you have high blood pressure and/or high cholesterol that requires further investigation/treatment. If so we will ask you to contact your GP to arrange this.
- The ultrasound test might reveal some thickening of the arteries that requires further investigation/treatment. If so we will ask you to contact your GP to arrange this.

9. What are the possible benefits of taking part?

You will have your blood pressure and cholesterol checked.

10. What if new information becomes available?

Sometimes during the course of a research project, new information becomes available. If new information becomes available that has implications for any treatment or investigation that you should receive we will contact yourself and your GP.

11. What if something goes wrong?

If you are harmed by taking part in this research and this is found to be due to someone's negligence, you have grounds for legal action and compensation. In addition if you have any complaints about a member of the study team we would ask you to inform another member of the study staff about your concerns.

12. Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the study will be kept strictly confidential. Any information that leaves the hospital will have your name and address removed. For this reason, on entering the study you will be assigned a unique patient number that will be used together with your sex and date of birth to identify study documents. If you agree, your GP will be informed of your participation in the trial.

13. What will happen to the results of the research study?

The results from the study may be published by a medical journal and/or presented to the regulatory authorities. This will be done confidentially and under no circumstances will your name be disclosed.

14. Who is organising and funding the research?

The study is being organised and funded by the Cardiovascular Division of King's College London and will be performed in the Department of Clinical Pharmacology, St Thomas' Hospital.

15. Who has reviewed the study?

The ethics for this study has been reviewed by St. Thomas' Hospital NHS Research Ethics Committee.

16. Contact for further information

If you have any further questions or queries about any aspect of the study at any time, then please do not hesitate to contact Prof. PJ Chowienczyk: Tel 0207 718-81502 (normal working hours). Thank you for considering taking part in the study. If you decide to take part in the study you will be given a copy of the subject information sheet and a signed consent form to keep.

APPENDIX 4 – PATIENT INFORMATION SHEET 3

INFORMATION FOR AND CONSENT OF PARTICIPANTS

**REnoVascular function Ethnicity Renin and
Endothelial Dysfunction**

Department of Clinical Pharmacology

St Thomas' Hospital London

CONFIDENTIAL

Ethnic Differences in Renovascular Function
Participant Information Sheet: L-NMMA study
(version 1.1 08-02-08)

1. Study Title

Ethnic differences in renovascular function

2. Invitation Paragraph

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

3. What is the purpose of the study?

Black African and Afro-Caribbean subjects have, on average, a greater risk of a stroke than white subjects living in South London. This may be because black subjects have higher blood pressure and arteries that relax less readily in response to stress than white subjects. These differences may, in turn, be due to greater retention of sodium by the kidney in black compared to white subjects. The purpose of this study is to compare blood pressure and arterial function in relation to kidney function in black and white subjects. This will help us to find out why black subjects are more at risk of stroke and will help us to prevent stroke in both black and white subjects.

4. Why have I been chosen?

You are being invited to take part in the study because you are a healthy volunteer at low risk of cardiovascular disease.

5. Do I have to take part?

It is up to you whether or not you want to take part. If you decide to take part you will be given this information sheet to keep and asked to sign a consent form. However, you are still free to withdraw at any time and without giving a reason.

6. What will happen to me if I take part?

If you decide to take part you will be asked to attend the department on one occasion. At this visit a cannula (small plastic tube) will be placed in a vein in your arm to allow us to give you a drug called L-NMMA. This drug temporarily stops the production of the substance nitric oxide that controls the relaxation of arteries and also the absorption of sodium by the kidney. L-NMMA is a drug that is commonly used for investigations of this sort. It is theoretically possible that you could have an unexpected reaction to L-NMMA but no adverse effects have been reported and it has been given to many hundreds of subjects. Before and after we give the L-NMMA we will make some measurements of the health of your arteries by using an ultrasound scanner to look at arteries in your arm. We will monitor your blood pressure and also the amount of blood your heart pumps by asking you to take a few breaths of an inactive tracer gas by breathing through a mouthpiece at regular intervals. We will also collect your urine for a period of 2 hours before and up to 2 hours after receiving the L-NMMA. This

means that the whole visit will take about 4 hours during which you will be lying on a bed able to read, listen to music or the radio.

7. What do I have to do?

You will need to lie still during the measurements.

8. What are the possible disadvantages and risks of taking part?

- You will have the discomfort of having the blood sample and cannula insertion.
- It is theoretically possible that you could have an unexpected reaction to L-NMMA but no adverse effects have been reported and it has been given to many hundreds of subjects.

9. What are the possible benefits of taking part?

You will be helping the advancement of scientific research.

10. What if new information becomes available?

Sometimes during the course of a research project, new information becomes available. If new information becomes available that has implications for any treatment or investigation that you should receive we will contact yourself and your GP.

11. What if something goes wrong?

If you are harmed by taking part in this research and this is found to be due to someone's negligence, you have grounds for legal action and compensation. In addition if you have any complaints about a member of the study team we would ask you to inform another member of the study staff about your concerns.

12. Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the study will be kept strictly confidential. Any information that leaves the hospital will have your name and address removed. For this reason, on entering the study you will be assigned a unique patient number that will be used together with your sex and date of birth to identify study documents. If you agree, your GP will be informed of your participation in the trial.

13. What will happen to the results of the research study?

The results from the study may be published by a medical journal and/or presented to the regulatory authorities. This will be done confidentially and under no circumstances will your name be disclosed.

14. Who is organising and funding the research?

The study is being organised and funded by the Cardiovascular Division of King's College London and will be performed in the Department of Clinical Pharmacology, St Thomas' Hospital.

15. Who has reviewed the study?

The ethics for this study has been reviewed by St. Thomas' Hospital NHS Research Ethics Committee.

16. Contact for further information

If you have any further questions or queries about any aspect of the study at any time, then please do not hesitate to contact Dr. Kenneth Connell, Department of Clinical Pharmacology Telephone 020 7188 4470 or mobile 07507492249. Thank you for considering taking part in the study. If you decide to take part in the study you will be given a copy of the subject information sheet and a signed consent form to keep.

APPENDIX 5 – DATA COLLECTION FORM – CROSS SECTIONAL STUDY

REnoVascular function, Ethnicity, Renin and Endothelial Dysfunction

REVERED CROSS SECTIONAL STUDY

PARTICIPANT: 11

Screening questionnaire: REVERED

Subject study number: REVERED 11			
Surname		Forename	
Sex	Male / Female	Age	yrs
DOB	/ /	Age of menopause (If applicable)	yrs

Contact details:

	PARTICIPANT	GP CONTACT
Tel: HmWk:		
Mobile:		
Email:		

What is your ethnic group? (2001 Census)

White

British.....☐

Irish.....☐

Any other white background (please state)☐

Black or Black British

Caribbean☐

African☐

<p>Please state your parent's ethnicity?</p> <p>Mother _____ Father _____</p>
<p>What is your country of birth? _____</p>
<p>How long have you lived in the UK? _____</p>
<p>Do you take any regular medications? _____</p> <p>_____</p> <p>—</p> <p>_____</p> <p>—</p> <p>_____</p> <p>—</p> <p>Exclude if taking regular medications.</p>
<p>Do you take any regular supplements? _____</p> <p>_____</p> <p>—</p> <p>_____</p> <p>—</p> <p>_____</p> <p>—</p>
<p>Do you take the contraceptive pill? YES <input type="checkbox"/> NO <input type="checkbox"/></p> <p>If yes, which one? _____</p>
<p>Do you take any inhalers? YES <input type="checkbox"/> NO <input type="checkbox"/></p> <p>If yes, which one? _____</p>
<p>Do you use recreational drugs? YES <input type="checkbox"/> NO <input type="checkbox"/></p> <p>Exclude</p>
<p>Have you been diagnosed with anxiety, depression, diabetes I or uncontrolled diabetes II, high blood pressure or any other specific chronic illnesses?</p> <p>_____</p> <p>—</p> <p>_____</p> <p>—</p> <p>_____</p> <p>—</p>

Have you been diagnosed with sickle cell disease?	YES <input type="checkbox"/> NO <input type="checkbox"/> Exclude
Do you have sickle cell trait?	YES <input type="checkbox"/> NO <input type="checkbox"/> DON'T KNOW <input type="checkbox"/>
Does anyone in your family suffer from sickle cell disease?	YES <input type="checkbox"/> NO <input type="checkbox"/> DON'T KNOW <input type="checkbox"/>
Are you pregnant?	YES <input type="checkbox"/> NO <input type="checkbox"/> Exclude
Have you given birth in the past 6 months?	YES <input type="checkbox"/> NO <input type="checkbox"/> Exclude
Are you a smoker?	YES <input type="checkbox"/> NO <input type="checkbox"/> Exclude
A non-smoker? (never smoked)	YES <input type="checkbox"/> NO <input type="checkbox"/>
Are you an ex-smoker?	YES <input type="checkbox"/> NO <input type="checkbox"/>
No. years stopped:	No. per day: No. of years: (if <6 months exclude)
How many units of alcohol do you consume per week? _____	Excl. if >28 units
♂	_____ Excl. if >24 units
♀	
<i>One unit = 1/2 pint of beer or 1 small glass of wine or 1 pub measure of spirit</i>	
ENTERED INTO THE STUDY?	YES NO

Data Collection Form

Date 1st Visit	Date 2nd Visit	Arrival Time:
Height cm	Weight kg	BMI kg/m ² (exclude if <18 or >36kg/m ²)
Waist circumference: cm	Hip circumference: cm	Waist: Hip Ratio
Last Day of Menstrual Cycle days		Departure Time:

(TIME.....) SEATED BLOOD PRESSURE

Seated BP	30 mins	35 mins	40 mins	Mean
SBP				
DBP				
HR				

(TIME:)PULSE WAVE MEASUREMENTS

Carotid-Femoral distance: cm
Subject study number: REVERED _____

Location:

(TIME STARTED:) Temp: **Room:** **Skin:**

FMD Filename

GTN

Filename.....Dose.....µg

Baseline start _____ (+ 1 min)

Baseline start _____ (+ 1 min)

Cuff up _____ (+ 5 min)

GTN given _____ (+ 5 min)

Cuff down _____ (5 min)

Stop acquisition

Stop acquisition

Baseline Dia:FMD%.....
dia:.....GTND%.....

Baseline

Flow	VTI	HR	Flow	VTI	HR
Baseline 1			RH 15 secs		
Baseline 2			RH 45 secs		
Baseline 3			RH 60 secs		
RH 5 secs			RH 75 secs		
RH 10 secs			RH 90 secs		

CIMT Measurements

	Left measur e 1	Left measur e 2	Left measur e 3	Left measur e Averag e	Right measur e 1	Right measur e 2	Right measur e 3	Right measur e Averag e
Diameter – Min (mm)								
Max (mm)								
IMT post(mm) Systolic								
Post (mm) Diastolic								

24 HOUR URINE

U Na	K	Prot/Creat:	Cortisol
Na:Creat.		K:Creat	(mRNA)
U Protein		Volume (ml)	Cortisone

FASTING LABS: (CHECK BOX)

Glucose:	mmol/L	T-Chol:	mmol/L
Creatinine:	μmol/L	HDL:	mmol/L
Urea		TG:	mmol/L
Urea		LDL	
PRA		Sodium	mmol/L
Aldosterone		Potassium	mmol/L

Daytime Systolic BP	Daytime Diastolic BP	Nighttime Systolic BP	Nighttime Diastolic BP	% Readings	Sleep Time	Awake Time

